

# **INVESTIGATION OF DIFFERENT EXTRACTION METHODS FOR HEMP SEEDS**

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by

**ABHIROOP MOOKERJEE**

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OR

Dean

College of Graduate and Postdoctoral Studies

University of Saskatchewan

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## ABSTRACT

Industrial hemp hearts were subjected to four different laboratory (research) scale solvent extraction techniques including soxhlet extraction, microwave pretreated soxhlet extraction, ultrasound extraction, and microwave extraction as well as extraction by non-solvent mechanical cold pressing. The maximum extraction yield of 54 % (w/w) was observed for both microwave as well as microwave pretreated soxhlet extracted seeds and the minimum run time of 30 minutes were noted for both ultrasound extraction and microwave extraction. The ratio of Linoleic acid to  $\alpha$ -Linolenic acid was found to be close to 3:1 which is considered to be optimal for nutrition. Also, the ratio of unsaturated fatty acids to saturated fatty acids was in the range of 6.7 to 9.1. Several minor compounds like  $\gamma$ -Sitosterol, stigmasterol, rhodoxanthin, carotene and methyl cholate were identified in the oils which have not been previously reported. Antioxidants  $\beta$ -Carotene and  $\gamma$ -Tocopherol have also been identified in the oil sample. Moreover, the acid value, iodine value and oxidative stability of the oils indicated that the oil was of an improved quality and that the level of unsaturation was very high. The poly unsaturated fatty acids (PUFA) found in the oils have numerous health benefits, the most important being improvement of cardiovascular health. A perfect combination of unsaturated fatty acids and saturated fatty acids and an appropriate medley of different antioxidants not only qualifies hemp seed oil as an excellent source of nutrition, but also as an important plant based (vegetable) oil for the higher value nutraceutical and pharmaceutical industries.

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## LIST OF ABBREVIATIONS

Acronym/Symbol	Name
DNA	Deoxyribonucleic acid
THCA	Tetrahydro Cannabinolic acid
CBD	Cannabidiol
THCV	Tetrahydrocannabivarin
THC	Tetrahydrocannabinol
THC-d3	Tetrahydrocannabinol d3
MeOH	Methanol
EtOH	Ethanol
HPLC	High Performance Liquid Chromatography
GC-MS	Gas Chromatography Mass Spectrometry
GC-FID	Gas Chromatography-Flame Ionization Detector
NMR	Nuclear Magnetic Resonance
IR	Infrared
US	Ultrasound Extraction
MW	Microwave Extraction
SE	Soxhlet Extraction
UASE	Ultrasound Assisted Soxhlet Extraction
MWPS	Microwave Pretreated Soxhlet Extraction
FFA	Free Fatty Acids
SFA	Saturated Fatty Acids

UFA	Unsaturated Fatty Acids
CP	Cold Pressing
PUFA	Poly-unsaturated Fatty Acid
MUFA	Mono-unsaturated Fatty acid
LA	Linoleic Acid
ALA	$\alpha$ -Linolenic acid
GC	Gas Chromatography
FID	Flame Ionization Detector
$\text{CCl}_4$	Carbon tetrachloride
KOH	Potassium Hydroxide
KI	Potassium Iodide
$\text{Na}_2\text{SO}_3$	Sodium thiosulphate
NaOH	Sodium Hydroxide
ASTM	American Society for Testing and Materials
AOCS	American Oil Chemists' Society
AOAC	Association of Official Analytical Chemists
AV	Acid Value
IV	Iodine Value
MC	Moisture Content
VP	Vapor Pressure
BP	Boiling Point



nd	Not detected
ACS	American Chemist's Society
HPEP	High Pressure Extraction Plant
I.D.	Internal Diameter
DPPH	2,2-diphenyl-1-picryl-hydrazyl- hydrate
ROS	Reactive Oxygen Species

# CHAPTER 1

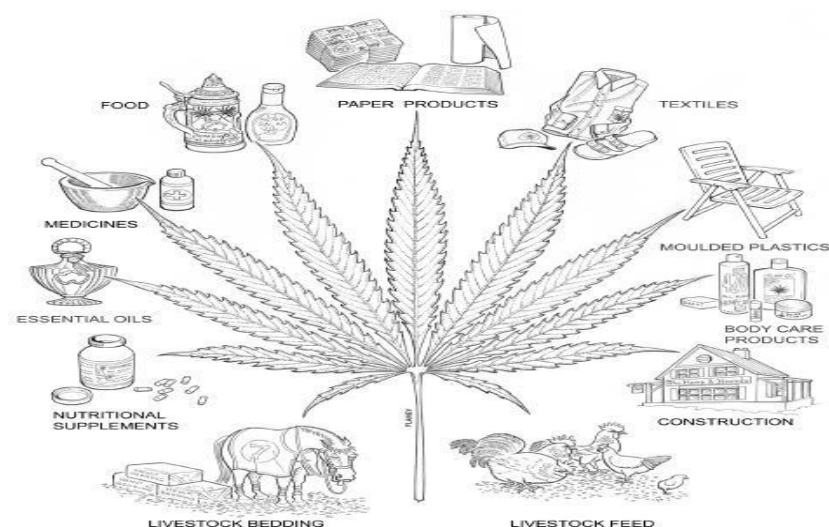
## 1.1. Introduction

*Cannabis sativa* is a widespread species in nature. It is known that Cannabis grows in a variety of climates and altitudes, mostly indigenous to the moist and swampy habitat of the Asiatic subcontinent. It also grows in the temperate regions as well as the alpine foothills of the Himalayas (Andre et al. 2016). The long coexistence between human beings and the plant led to its domestication and further cultivation. Hemp is a variety of *Cannabis sativa* with lower composition of the psychoactive compound, THC (>0.3%). On the other hand, it is rich in another bioactive compound called cannabidiol (CBD). Nowadays, hemp is being used industrially for the manufacture of rope, textiles, clothing, shoes, food, paper, bioplastics, insulation, and even biofuel.

**Table 1.1: The fields in which different parts of hemp has profound uses (Small and Marcus 2002)**

Seeds	Long fiber	Woody stem core	Female floral tract	Whole plant
Confectionary, baked goods, salad, oil, cosmetics, animal food, gamma linolenic dietary supplements	Plastic-molded products, specialty papers, construction fiber board, biodegradable landscape matting and plant culture products, coarse textiles, fine textiles	Animal bedding, thermal insulation, construction	Medicinal cannabinoids, Essential oils, insect repellent	Alcohol, fuel silage

One of the most important parts of the hemp plant for its use in the nutraceutical and food industry is the seed. This is because hemp oil is believed to contain the ideal ratio of omega 6 and omega 3 fatty acids (Callaway, 2004). Linoleic acid (LA) and alpha-linolenic acid (LNA) are present as the major omega-6 and omega-3 polyunsaturated fatty acids (PUFA), respectively (Leizer et al. 2015) in hemp seed oils.



**Figure 1.1: Different industries where hemp is used in a pictogram (Andre et al. 2016)**

The level of PUFA in hemp is considered to be the highest for any vegetable oil (80-81%) (Pate and Ranali 1999). Previous reports have stated that the ideal ratio for the consumption of omega-6 and omega-3 fatty acids by human beings must be between 2.5 and 5.0 (Callaway 2004). A study comparing the fatty acids of two different varieties of hemp has shown that the ratio of omega-6 and omega-3 fatty acids is in the range of 2.5 to 2.7 (Callaway 2004). Additionally, it is known that the presence of gamma-linolenic acid (GLA) in hemp seed oil makes it somewhat nutritionally unique compared to most seed oils. Essential fatty acids are important in pathogenesis, in the prevention of coronary heart disease, hypertension, breastfeeding and other physiological functions. The essential fatty acids and  $\alpha$ -linolenic acid present in the oils are important ingredients in formulating products for the cosmetics and wellness industries (Pate and Ranali 1999).

Antioxidants are recognized for their potential in health promotion and prevention of aging-related diseases as well as in the prevention of cancer and heart diseases. Antioxidant property of cold pressed hemp seed oil was proven to be more effective in free radical elimination

than extra-virgin olive oils (Ramadan et al. 2006; Tubaro et al. 1998). Apart from the nutrient value of the fatty acid content in hemp seeds, it contains other important compounds like gamma-sitosterol, beta carotene and salicylic acid. which complements the nutritional composition of the oil and makes it functionally more effective.

It is of utmost importance in the plant-based oil industry to determine the total fat composition since the price of the raw material is a function of its richness in the final product sold to the customers. It has been reported that the most common practice and laboratory method involved in oil extraction is the conventional soxhlet extraction apparatus which is inexpensive but time consuming and requires the use of chemical solvents to extract the oil. A laboratory or pilot scale Soxhlet extractor may be used by the oilseed industry to simulate a continuous commercial scale solvent extractor. Cold pressing is a popular mechanical method for extraction of hemp seed oil and is commonly used for commercial production of the oil. Cold pressing is advantageous since it is a low temperature extraction without the use of any organic solvent, thus reducing environmental and health hazard concerns. Moreover, the low temperature extraction preserves the thermolabile compounds present in the hemp seeds. On the other hand, it is disadvantageous since a higher quantity of residual oil remains in the cake resulting in a relatively lower extraction efficiency (Aladic et al. 2015). Supercritical fluid extraction is considered a green technology and for some high-value oils it may be an alternative to conventional industrial processes such as pressing or solvent extraction. The supercritical fluid extraction (SFE) technique has benefits which include low temperature extraction which preserves thermally sensitive components in the oil, it does not use organic solvents, and by adjusting extraction parameters it can produce high quality oil extracts. SFE however, is a batch extraction method and may be higher cost for the extraction of large commercial volumes of seed.

In order to find a solution to the above-mentioned problems of using solvent extraction, some novel methods have been reported; microwave assisted, and ultrasound assisted extractions are few of them. These methods are being recently used as an alternative extraction method to obtain oil from seeds including hemp (Jiao et al. 2014). The advantages of these methods are that they may offer high extraction efficiencies at reduced extraction time, temperature and higher throughput capacities. They promote extraction by breaking down the cell walls of the structures containing the oils. They can also lead to the extraction of essential nutraceutical and antioxidant compounds with lesser degenerative effects on the cells.

Herein, we report comparative extraction efficiencies of different oils recovered from hemp hearts using traditional and modified oil separation strategies for maximum yield. Further, the physiochemical parameters have been measured to analyze the fatty acid and antioxidant profile of the extracted oils for several therapeutic applications.

## **1.2. KNOWLEDGE GAP**

- 1.** Most of the previous studies have made a comparison between either the extraction techniques which uses high temperatures for oil extraction or the ones that use low temperatures for extraction to compare the yields. Very few studies have made a clear comparison between the different extraction techniques which uses wide temperature ranges for extraction.
- 2.** Hemp seed oil contains various thermolabile compounds which are preserved at low temperatures. As a result, the extraction technique affects the quality of oil in terms of its physico-chemical characteristics to a great extent. There is a dearth of knowledge as to how a wide range of extraction techniques affect the quality and physico-chemical properties of the obtained oils.
- 3.** Although previous researches have compared the lipid and antioxidant profile of the

extracted oils and most of them have led a foundation for future research none of them have specifically mentioned the application where the oils can be used.

### **1.3. HYPOTHESIS**

1. The amount of oil yielded by different extraction techniques like the Soxhlet extraction, microwave pretreated Soxhlet extraction, ultrasound extraction, ultrasound assisted Soxhlet extraction, microwave extraction, cold pressing and supercritical fluid extraction operated using optimal parameters and conditions are different.
2. The FA (fatty acid) and antioxidant profile as well as the physico-chemical properties of hemp seed oil extracted by different extraction technique is different for different techniques.
3. Due to the presence of antioxidants and fatty acids in a desirable ratio, the oil can be used in nutraceuticals as well as in the medical and pharmaceutical industries.

### **1.4. RESEARCH OBJECTIVES**

The specific objectives of my research include:

1. Extraction of hemp heart oils using different extraction techniques like the conventional Soxhlet extraction, microwave pretreated Soxhlet extraction, microwave extraction, ultrasound extraction and cold pressing to find the technique producing maximum yield.
2. Characterization of the extracted oils using different analytical techniques like the GC-MS and NMR to understand the fatty acid and antioxidant profile of the oils obtained by each extraction technique and analysis of their physical and chemical properties.
3. Comparison of key processing parameters for its final quality, extraction time, efficiencies, and areas of application.

## CHAPTER 2

### 2. LITERATURE REVIEW

#### 2.1. Taxonomy for Cannabis

The nomenclature of cannabis has been severely critiqued and underwent numerous nomenclatural changes (Pollio 2016). Although Jean-Baptiste Lamarck proposed that there were two species of cannabis—*Cannabis sativa* and *Cannabis indica*, it was rejected over the years as genomic DNA studies came into action. More recently, a biphasic approach, i.e. combining both the morphological and chemical characteristics was adopted by two scientists—Small and Cronquist (1976) to classify cannabis into four taxa, all belonging to the single species *Cannabis sativa*. These are as follows:

- *Cannabis sativa* L. subsp. *sativa* var. *sativa*
- *Cannabis sativa* L. subsp. *sativa* var. *spontanea* Vavilov
- *Cannabis sativa* L. subsp. *indica* Small & Cronquist var. *indica* (Lam) Wehmer
- *Cannabis sativa* L. subsp. *indica* Small & Cronquist var. *kafiristanica* (Vavilov) Small & Cronquist.

#### 2.2. Morphology of hemp (*Cannabis sativa* L.)

Industrial hemp is a short day, temperate climate, annual plant. The hemp seed looks like a popcorn. It is kernel shaped, looks brown in color and typically under 4 mm in size. The weight of a kernel weighs between the range of 0.013 g to 0.021 g depending upon the variety of the plant from which it is coming. The weight of an average a bushel of hemp seed is around 20 kg (Techfibre Industries 2017). The fruits also called the seeds that are formed almost entirely as a

result of the ripening of the ovary. The ovaries are enclosed by bracts subtending the pistils unless the fruit matures. Hemp seeds develop with a tough outer hull which protects the seed until the conditions are ideal for germination. This hull helps the seed survive for a long period of time under unfavorable conditions such as in a wild and unpredictable environment. Hulled seeds are those which have the outer hull removed whereas the unhulled (whole) seeds are the ones which has the hull intact. Approximately, a bag of unhulled seeds has around 40% less seed per pound compared to a bag of hulled seeds.

**Table 2.1: Nutritional composition content (%) of hempseed (Callaway 2004)**

	<b>Whole seed</b>	<b>Seed meal</b>
<b>Oil (%)</b>	35.5	11.1
<b>Protein (%)</b>	24.8	33.5
<b>Carbohydrates (%)</b>	27.6	42.6
<b>Moisture (%)</b>	6.5	5.6
<b>Ash (%)</b>	5.6	7.2%
<b>Energy (kj/100 g)</b>	2200	1700
<b>Total dietary fiber (%)</b>	27.6	42.6
<b>Digestible fiber (%)</b>	5.4	16.4
<b>Non-digestible fiber (%)</b>	22.2	26.2

### **2.3. Hemp and its uses**

*Cannabis sativa* can be considered as a multi-purpose plant that has been domesticated in the early years. The bast fiber of the stem and oil content in the seeds can be used for multiple purposes. The epidermal glands of the plant also secrete an intoxicating resin. Historically, the name hemp



has been used primarily for the fiber cultigen and its fiber preparations, and marijuana for the drug variety and its drug preparations (Small and Marcus 2002). Till mid-nineteenth century, there was a rivalry between hemp and flax as to which was the chief textile fiber of vegetable origin, after which hemp was described as “the king of fiber-bearing plants,—the standard by which all other fibers are measured” (Boyce 1900).

Popular Mechanics magazine (1938) crowned hemp as “the new billion-dollar crop,” stating that it “can be used to produce more than 25,000 products, ranging from dynamite to Cellophane.” It has been estimated that hemp fiber has the potential to replace other biological fibers in many applications and can sometimes compete with minerals such as fiber, steel and glass. Hemp bast fibers are used as a substitute of glass fibers. They are also used in the automotive industry to produce bioplastics because of their high tensile strength and light weight. Moreover, considering their anti-bacterial properties, it is assumed that they have been used to make surgical instruments or anti-bacterial finishing agents (Andre et al. 2016). Cannabinoids obtained from hemp seeds and inflorescence also have various pharmacological properties as stated by Andre et. al. (2016).

Hemp seed oil is believed to contain typically over 90% unsaturated fats. It is a very rich source of two essential fatty acids- linoleic acid and alpha linolenic acid. The ratio between *omega*-6 and *omega*-3 (n6/n3) is usually between 2:1 and 3:1 in hempseed oils, which is considered ideal for human nutrition. Gamma linolenic acid and stearidonic acids are also present. Dietary fatty acids present in hempseed meal can be used for the treatment of tuberculosis without the use of antibiotics. Moreover, the fatty acid profile of hempseed oil has been considered to be remarkably similar to that of black current seed oil which might mean that it has a beneficial impact on the immunologic vigor (Callaway 2004). Albumin, a globular protein, and edestin, a legumin, are the two main proteins in hempseed and both are rich in the amino acids that are essential to human

health. The pressed cake (after the extraction of oil) is also highly rich in proteins, around 33.5% and dietary fibers (42.6 %) (Callaway 2004). According to Wang and Xiong (2019), hempseed proteins contain a well-balanced combination of all the essential amino acids and its amino acid profile can be compared to other high-quality protein sources like soy (Tang et al. 2006). The protein digestibility of hemp seed proteins was also found to be comparable to those of casein (House et al., 2010). Various researches have reported the edible nature of hemp proteins and its use in bakery (Pojić et al. 2015; Ruban et al. 2016), extruded products (Norajit et al. 2011), dairy and infant formula (Dabić et al. 2018), processed meat and beverages (Naumova et al. 2017). Apart from that, the unprocessed meal is also an important animal fodder. Although, hemp seeds are an emerging source of proteins and are becoming more common in the food and nutraceutical industries, various aspects of the protein like its role in food processing, areas of application and structure-functionality relationship needs more research and exploration.

#### **2.4. Availability of hemp in Canada**

According to the 2018 reports by Health Canada, 77,800 acres of industrial hemp were planted in Canada out of which 33,000 acres (38.5 %) were planted in Alberta, 27,100 acres (35 %) in Saskatchewan and 11,500 acres (about 15%) in Manitoba. It is estimated that roughly 90% of the total seed for hemp cultivation is produced in Canada which contains mostly Canadian developed varieties. The yield of hempseeds typically reaches about 1,000/lb/acre on dryland which is equivalent to approximately 22 bushels/acre on a 44-pound bushel and up to 3,000/lb/acre on fields that are irrigated. According to industry estimates, it has been reported that certified organic hemp production is growing in trend and is reaching approximately half of the total production. There are approximately 12 to 14 hemp processing companies in Canada, most of them being in the Prairie region.

**Table 2.2: Composition (%) of Fatty acids present in hemp seed and its comparison with other oils (Callaway 2004)**

Seed	Palmitic Acid (%)	Stearic Acid (%)	Oleic acid (%)	Linoleic Acid (%)	$\alpha$ -Linole nic acid (%)	% PUFA	n6/n3 ratio
Oil	5	2	9	56	22	84	2.5
hempseed							
Fiber	8	3	11	55	21	77	2.7
hempseed							
Black	7	1	11	48	13	81	4.1
currant							
Flax(linseed)	6	3	15	15	61	76	0.2
Evening	6	1	8	76	0	85	>100
primrose							
Sunflower	5	11	22	63	<1	63	>100
Wheat grain	3	17	24	46	5	56	10.2
Rape seed	4	<1	60	23	13	36	1.8
Soy	10	4	23	55	8	63	6.9
Borage	12	5	17	42	0	66	>100
Corn	12	2	25	60	1	60	60.0
Olive	15	0	76	8	<1	8	>100

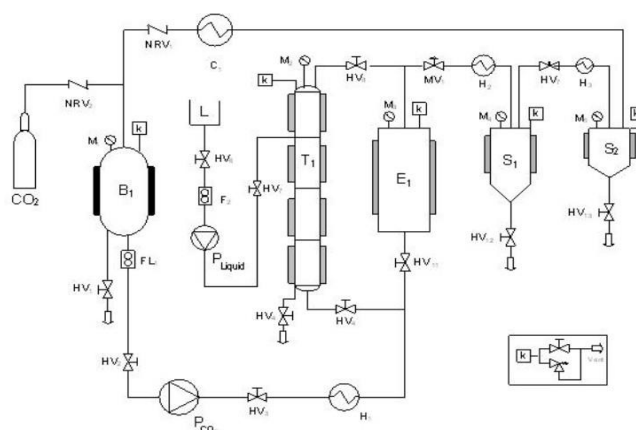
## 2.5. Extraction Techniques

### 2.5.1. Supercritical Fluid Extraction

According to Aladic et al. (2014), near complete extraction of the residual hemp oil from the pressed cake (after cold pressing) was achieved by supercritical CO<sub>2</sub> method with the obtained oil also having higher tocopherol content when compared to cold pressed oils. Furthermore, it was reported that supercritical method included low temperatures of extraction, consumption energy, higher quality of the end-product and solvent free extracts when compared with other traditional methods of extraction such as the Soxhlet extraction.

Aladic et al. (2015) also suggested that supercritical CO<sub>2</sub> extraction was proven to be more effective than Soxhlet extraction using pressurized n-propane for extraction of hemp seed oil. It was shown that supercritical fluid extraction (SFE) technique has many advantages over traditional methods, especially in the preservation of thermosensitive compounds (using low extraction temperatures) and reduced energy consumption. Another major advantage of this method is the production of solvent free extracts.

Jokic et al. 2013 performed supercritical CO<sub>2</sub> extraction in a laboratory scale high pressure extraction plant (HPEP). The most abundant fatty acids obtained was linoleic acid (>50%). Oleic and linoleic acids were also obtained in abundant quantities as reported by them. These results indicated that soybean oil is rich in PUFA (poly unsaturated fatty acid). Moreover, no fatty acids with chain length shorter than C14 or longer than C24 was detected.



**Figure 2.1: Flowsheet of a pilot scale apparatus (Da Porto et al. 2012)**

Extraction experiments were also performed by Grijo et al. (2019) and Da Porto et al. (2012) using supercritical Carbon dioxide with ground and garbled hemp seeds. The flow rate was maintained at 10 kg/h. The independent parameters in each of the experiments were temperature, pressure and solvent passed through the extractor. Temperatures in each of the experiments were maintained

within a range of 40 °C to 80 °C, pressure 200-400 bar and the solvent flow rate varied between 20 and 60 kg CO<sub>2</sub>/kg feed. The extraction yields and composition of hemp seed oils obtained by Grijo et al. (2019) using supercritical CO<sub>2</sub> were similar to previously reported literatures, however, the tocopherol content obtained in his oils was higher than previously reported. It was also shown that cost effectiveness of using pressurized n-propane over supercritical CO<sub>2</sub> was more. Hempseed oil can find use as a non-traditional dietary source of oil rich in essential fatty acids and antioxidants for both promotion of health and prevention of diseases was proven in the research by Da Porto et al. (2012).

### **2.5.2. Conventional Soxhlet extraction**

This technique is a widely used method by the researchers for the extraction of oils from seeds, but the drawback lies in the fact that it is time consuming as well as non-eco-friendly due to the large volumes of organic solvent released in the atmosphere. Wong et al. (2014) used traditional Soxhlet extraction using ethanol for the extraction of essential oils from cinnamon leaves. It was reported that ethanol was less harmful compared to other solvents like methanol or chloroform and produced a better yield compared to organic solvent extraction.

An improvised version of the traditional Soxhlet extraction method was given by Luque-Garcia et al. (2004) which would retain all benefit and advantages of the original method like solvent contact with the fresh sample during the entire extraction step, elimination of the filtration step as well as any sort of manipulation and also narrow down the shortcomings like excess time and environmental pollution. Sunflower, soybean and rape seeds for estimating the oil content were used.

A modified method of the conventional Soxhlet extraction was developed by Subramanian et al. (2011) which involved increasing the number of operating cycles of the experiment thereby

reducing the operating time as well as energy involved. They introduced a simple modification in the traditional Soxhlet apparatus in such a way that it contained a double bypass sidearm (DBSA) instead of one bypass sidearm. Upon heating of the flask during experiment, double the volume of the vapors directly reached the extraction tubes thereby reducing the number of cycles required as well as the extraction time. Apart from that, the entire experiment was meant to take place at a lower temperature, thereby preserving the thermolabile compounds present in the oil. From the results of Subramanian et al. (2011), it was concluded that DBSA had advantages over regular Soxhlet extraction in terms of extraction times (shorter) and solvent consumption (lower). Moreover, it was proposed that this method was a simpler and convenient method and could be used for other extractions.

Ibemesi et al. (1990) reported that the maximum oil extraction yield from rubber seed was achieved near the boiling point of the used solvent. Again, in order to optimize the number of cycles to be used, a dark colored paper was kept behind the Soxhlet apparatus. A colored appearance of the solvent falling through the nozzle of the Soxhlet apparatus implied that the Soxhlet extraction was continuing, whereas a colorless appearance of the solvent meant complete extraction. In this way, the number of stages for the Soxhlet apparatus can be optimized.

### **2.5.3. Percolation method:**

Counter current extraction of oils from ground seeds (rapeseed, sunflower and soybean) using percolation techniques was studied by Avram et al. (2014). Comparison of his results were made with traditional Soxhlet extraction. Analysis of the time evolution curves of the extraction yields showed that they were of similar shape which concluded that the extraction yield was not dependent on the solid phase type of the oleaginous material and the solid-liquid contacting procedures. However, the extraction yield was greatly influenced by the particle size of the seed

and moderately by the temperature of the reaction.

#### **2.5.4. Steam distillation**

This method was studied by Boutekedjiret et al. (2003) for the extraction of essential oils from rosemary plant in his research. Similarly, coriander oil was extracted from the leaves using a similar approach by Anitescu et al. (1997). Also, this method was utilized by Ammann et al. (1999) in his comparative study of peppermint oil extraction. Although this method is not applicable for the extraction of oils containing triacylglycerols or “fixed” oils (eg. hemp seed oil), it is a very essential method for the extraction of essential oils which mostly comprises of terpenes and volatile lipids such as aldehydes.

#### **2.5.5. Superheated water extraction**

If the temperature of liquid water is raised under pressure (50 atm - 100 atm) between 100 °C and 374°C, there is a sharp decrease in the polarity, and it can be used as a solvent for extraction of various analytes (Smith 2002). The advantage of the method is that it has shown results comparable to organic solvent extraction (Soxhlet extraction) and is also a clean and green method of extraction since it does not use any organic solvents for extraction. According to Van Bavel and his team, superheated water at 300 °C and 50 atm pressure showed comparable results with solvent extraction for the extraction of naphthalene from industrial oil (Van Bavel et al. 1999).

Peppermint oil extraction by super-heated water extraction was studied by Ammann et al. (1999) which also showed comparable results to conventional soxhlet extraction. The procedure was very similar to steam distillation.

#### **2.5.6. Cold pressing**

Cold pressed hemp seed oils were obtained by Jokic et al. 2013 by pressing 1 kg of hemp seeds in a mechanical screw expeller using a specific set of parameters.

The same technique was availed by Aladic et al. (2014) to extract oils from hemp seeds. Several process parameters such as nozzle size, temperature and frequency were studied in his experiments. Monitoring of the oil recovery and quality of the obtained cold pressed oil was maintained after a set of experiments optimized using RSM. Promising results were obtained since the experimental conditions had an impact on the quality and yield of oil. The maximum yield of oil took place at a pressing temperature of 60 °C, frequency of 20 Hz and a nozzle of ID 6 mm.

Investigations of the effects of cold pressed extraction on the total phenolic content of grape seed oils to optimize the oil yield were made by Rombaut et al. (2015). Grape seeds obtained from different harvest times were dried to 7% moisture content at a temperature of 40°C. Materials and process parameters effect was determined by Taguchi experimental design. The variables considered were pre-heating temperature, rotational speed (20-110 rpm) of the screw and restriction die size (8, 10, 12 and 15 mm). Obtained oils were centrifuged at 3000 g for 10 minutes and moisture, ash, total phenolic contents were determined. The author concluded that the most effective parameter which determined the grape seed oil phenolics was the type of the seeds. Optimization of the device conditions led to an increase in the number of phenolic compounds and on the contrary, an increase in the initial moisture content of the raw material led to its decrease. The yield of oil ranged between 48.9-73 % with the maximum yield of 73% being achieved for a preheating temperature of 90 °C , a dye diameter of 15 mm and screw rotation speed of 40 RPM.

#### **2.5.7. Organic solvent extraction**

It is a type of Liquid Solid Extraction (LSE) in which an organic solvent is used for the extraction of compounds from a solid sample. It is stated that both types of solvents that are immiscible or miscible with water can be used for the purpose of extraction. Some common examples of organic solvents that can be used are methanol, acetonitrile, hexane and chlorinated



solvents (Moldoveanu and David 2015). Subratti et al. 2019 used liquid Dimethyl Ether (DME) for the extraction of hemp seed oils. It was reported that the extraction yields were notably higher than other organic solvents used like n-hexane. It was also stated in the paper that DME evaporated rapidly at room temperature without leaving any residual solvent behind unlike other solvents which required the use of heat and reduced pressure to be removed from extracts. Moreover, it was a green solvent and hence much safer than other organic solvents (Subratti et al. 2019).

#### **2.5.8. Ultrasound/Ultrasonic assisted solvent extraction**

This process is believed to extract the total fat content from oleaginous seeds like sunflower, rape and soybean. A method was proposed by Luque-Garcia et al. (2003) in which the Soxhlet chamber was placed in a thermostat bath and ultrasound were applied through it using an ultrasonic probe. This coupled effect of conventional Soxhlet extraction along with ultrasound extraction led to an increased oil yield.

Ultrasound waves were utilized by both Rezvankhah et al. (2019) and Lin et al. (2010) to extract oils from hemp seeds. For experimental purposes, an ultrasonic probe (diameter 12 mm) having an output power of 400 W and operating at a frequency of 20 kHz were taken into consideration by Rezvankhah et al. (2018). The ultrasound run times used in the experiment were 10, 20 and 30 minutes and the powers used were 20, 60 and 100 Watts. The solvent chosen was n-hexane and the temperature of the solvent-seed mixture was maintained constant at 25 °C using an ice bath. A cycle of 7 seconds on and 3 seconds off was chosen as the pulse in all the experiments. After centrifugation, the solvent was separated from the solute through a filter paper by a vacuum pump. Thereafter, the solvent-oil mixture was separated using a rotary vapor under a reduced pressure at a temperature of 50 °C.

The effect of ultrasound power and time on the extraction yield of the oils as obtained from his experiments are illustrated in Table 2.3.

**Table 2.3: Effect of power and time on extraction yield according to Rezvankhah et al. (2019)**

Run	Power (W)	Time (min)	Yield (meq/kg)
1	20(-1)	10(-1)	31.94,31.79
2	100(+1)	10(-1)	33.68,33.58
3	20(-1)	30(+1)	33.80,34.07
4	100(+1)	30(+1)	34.48,34.80
5	20(-1)	20(0)	33.54,33.40
6	100(+1)	20(0)	34.90,34.66
7	60(0)	10(-1)	33.52,33.74
8	60(0)	30(+1)	36.10,35.49
9	60(0)	20(0)	34.70,35.09
10	60(0)	20(0)	35.50,35.09
11	60(0)	20(0)	34.80,35.09
12	60(0)	20(0)	35.40,35.09
13	60(0)	20(0)	34.70,35.09

Experiments using hexane as a solvent, a fixed operation frequency of 20kHz, solvent to solid ratios of 1:1, 5:1, 7:1, 10:1 and 15:1 mL/g, cycles of 20:10, 20:20, 20:30, 20:40, 20:50 s/s and varying ultrasonic powers of 100 W, 150 W, 200 W, 250 W and 300 W were carried out by Lin et al. 2012. An increase in the oil yield with an increase in ultrasonic time was observed for both the studies. It was found that the maximum oil yield was achieved at a power of 91 W for 10 min and an increase in the ultrasonic time and power tend to cause oxidation in the obtained oils (Rezvankhah et al. 2019). It was concluded that an extraction time of 30 min with an ultrasound power of 200 W was enough to obtain maximum oil yield. A considerable increase in the extraction

yield was also noticed upon increasing the solvent to solid ratio of the oils. Finally, a conclusion can be drawn that ultrasonic extraction is an excellent and safe method for the preservation of phytochemicals for the food, pharmaceutical and cosmetic industry.

## **2.6. Different heat pre-treatment methods for hemp seeds**

The influence of different heat pre-treatment methods on the quality of the obtained extracted vegetable oils was investigated by Veldsink et al. (1999). According to the author, different techniques of heating rely on different heat transfer mechanisms like conduction, convection or radiation. While steaming or oven cooking are based on the combined effects of conduction and convection, heating by electromagnetic such as infrared (IR) and microwaves (MW) follow the principles of radiation. The following heat treatments were deployed- live steam at 3.0 bar and 402 K, an industrial continuous microwave (6 kW and 2450 MHz) and an IR oven (short wave radiators at 513 K and 40% output, microwave radiators at 498 K and 70% output). Radiation seemed to be more effective for pre-treatment compared to normal oven heating methods in terms of both time and energy consumption as well as quality-improving characteristics and hence can be used in the industrial scale for pre-treatment purposes.

## **2.7. Effect of microwaves on the seed cell walls**

The influence of microwave heating on sunflower seeds was studied by Anjum et al. (2006) and the first ever theoretical model describing the cell rupture mechanism for microwave assisted extraction (MAE) of bioactive compounds from plant samples was presented by Chan et al. (2016).

Chan et al. (2016) showed that heat pre-treatment of the seeds does not degrade the quality of the oil produced. Despite obtaining the best results by infrared heating, it could not be considered as the ideal method since the seeds get burnt (upon exposure to such high temperatures)

and expel heavy odor. On the other hand, it was reported that microwave pre- treatment increases the oxidative stability of the oil as well as enhances the level of free fatty acids (FFA), phosphorus and iron in the oil.

**Table 2.4: Comparison of some results of different forms of pre-treatment for oilseeds**

<b>Parameter</b>	<b>Control oil</b>	<b>Microwave</b>	<b>Infrared</b>	<b>Steam</b>
FFA [%]	0.92	0.96	0.78	1.10
P (ppm)	50	257	27	72
Fe (ppm)	0.11	3.60	0.38	1.51
PV	0.6	0.3	0.3	0.2
E 232/268	1.15/0.14	2.03/0.93	1.26/0.35	1.63/0.72
Tocopherols	752	791	795	828
Rancimat	5.3	38	14.5	32

The effect of traditional oven radiation with microwave radiation on the physicochemical properties of corn seed oil were compared by Zheng et al. (2018). The researchers concluded that microwaves heat the intracellular moisture within the plant cells consistent with Lambert's law. The plant cells stretched and ultimately ruptured when the elastic limit of the cell wall was reached as a result of the heat energy generated. The author's model showed that cell rupture mechanism was dominant in MAE of bioactive compounds from plants. The energy required to rupture plant cells by the intracellular moisture primarily depended upon the mechanical property of the cell wall in such a way that energy required was high for plant cells with thick walls and of high shear modulus. It was shown by Tsubaki et al. (2017) that microwaves penetrate inside the biomass substrates generating heat from within which enhanced the extraction efficiency of the components

contained in the plant bodies.

## **2.8. Analysis of extracted oils:**

Gas Chromatography was used by Jokic et al. (2013) for the determination of fatty acid composition in soybean oil. GC-MS was also utilized by Orhan et al. (2000) in order to analyze the fatty acid composition of hemp seed oils. The separation was performed on a column Forte GC 30 m length, 0.25 mm inner diameter and film thickness 0.25  $\mu\text{m}$  with the injected sample volume being 1  $\mu\text{l}$ . The operating conditions included a split injection ratio 30:1, with the inlet temperature being set at 498 K, the detector temperature set at 553 K. The carrier gas used was He at a flow rate of 0.8 ml/minutes. Gas Chromatography-Mass Spectrometric analysis were also performed by Rezvankhah et al. (2018), (2019) and Lin et al (2012) to analyze the oils obtained by ultrasound assisted extraction of hemp seed oils. Measurement and quantification of the different fatty acids present in hemp seed oil using Gas Chromatography were done by Oomah et al. (2002). GC-FID analysis was used by Blade et al. (2005) for assessing the compositions of fatty acids from hemp seed oil obtained from the prairie regions of Canada. IUPAC standard methods for the analysis of Fatty acids present in hemp seed oils was followed by Paz et al. (2014). In his experiments, the usual procedure was followed by column chromatography following a method adopted by the European Union.

A GC system which was equipped with a Flame Ionization Detector and a capillary column (length= 100 m, Internal diameter= 0.22 mm and External diameter= 0.33 mm) was used by Rezvankhah et al. 2019. High Performance Liquid Chromatography (HPLC) analysis of soybean oil triacylglycerols was performed using a Perkin-Elmer High Performance Liquid Chromatography system series 200. GC-MS analysis of hemp seed oils was also made by Aladic et al. (2015). A gas chromatograph with a capillary column HP88 100 m long with a diameter of

0.25 mm and a stationary phase 0.20 microns thick; a split-splitless injector (temperature 250 °C) and a flame-ionization detector (temperature 280 °C) were used for the determination of fatty acids in his experiments. The characterization of oils developed from hemp seed meal can also be determined by Folch's extraction principle according to Pojić et al. (2014).

Also, six samples of cold pressed hemp seed oils were studied by NMR and FT-IR (Siudem et al. 2019). A combination of <sup>1</sup>H NMR and <sup>31</sup>P NMR spectroscopy and multivariate statistical analysis to classify 192 samples from 13 vegetable oils like hazelnut, sunflower, soybean, walnut, coconut, virgin olive and other oils from various regions of Greece was taken into consideration by Vigli et al. (2003).

Optimization of the <sup>1</sup>H NMR method was incorporated by Castejon et al. (2014) in order to determine the fatty acid types with the same accuracy as the GC. In this research, the authors compared and analyzed different Spanish extra-virgin olive oils. They then compared the results (obtained from the NMR) with the GC-FID and both the results showed good correlation.

A different approach to estimate the amount of free fatty acids in lipids was put forward by Kumar et al. (2011). A comparison was made between the integrals of the <sup>1</sup>H signal at 2.3 ppm which was due to both free fatty acids and esterified fatty acids and the integral of the signal at 4.1-4.4 ppm which was due to the glycerol upon esterification. The method concluded that the subtraction of signals considering the number of protons responsible for each signal gave the integral of free fatty acids from which the concentration of each fatty acid could be calculated.

On the other hand, Medina et al. (1994) demonstrated the use of <sup>13</sup>C NMR to quantify free fatty acids in fish oil using integrals of the carbonyl region. Free fatty acids have carbonyl resonances in the region 178–176 ppm. The carbonyl resonances of the esters are in the 174–172 ppm range. The details in the <sup>13</sup>C NMR spectrum allowed to conclude that there was preferential

cleavage of docosahexaenoic acid (DHA) upon thermal treatment of canned fish. He also concluded that the NMR is better than conventional methods, since sample pretreatment may lead to oxidation and loss of PUFA.

More recently the application of  $^{13}\text{C}$  NMR and  $^1\text{H}$  NMR on the analysis of Fatty acids present in oils and lipids were studied by Lankhorst and Chang (2018).

## **2.9. Assessment of Physical and Chemical properties**

### **2.9.1. Viscosity:**

The viscosity of a fluid is defined as its resistance to deformation at a given rate. In other word, it can also be defined as the internal friction of a fluid. A viscometer gives the fluid's resistance to "flow"(Leblanc 1999). Diamante and Lan (2014) measured the viscosity of several vegetable oils like canola, peanut, grapeseed, rice bran, olive, walnut and sunflower oils using a rotational viscometer with a coaxial cylinder. Their study reported that all the vegetable oils were Newtonian in nature and among the different oils, rice bran oil had the maximum viscosity (Diamante and Lan 2014).

### **2.9.2. Acid value**

Acid value is defined as the number of mg of KOH which is necessary to neutralize the FA present in 1 g of the sample. It is a measure of the amount of FFA that is present in the oil. The acid value of hemp seed oils was determined by Latif et al. (2009) using AOCS official methods. Moreover, Alajtal et al. (2018) measured the acid value of some vegetable oils using both the AOAC and AOCS methods.

### **2.9.3. Iodine value**

Iodine value is a measure of the unsaturation present in fats and oils and is expressed in terms of the number of grams of iodine absorbed per 100 gram of the oil. The iodine value of hemp seed oils using AOCS official methods was determined by Latif et al. (2009). It was noted from the above study that the iodine value was between the range of 150-160 g of iodine/gram of solution as was reported previously. Apart from that the iodine value was measured by Alatjal et al. (2018) using both the AOAC and AOCS methods.

### **2.9.4. Oxidative stability**

The term oxidative stability determines the resistance of the oils and fats to oxidation. Oxidation stability is one of the major quality parameters of edible vegetable oils since it determines their usefulness in technological processes as well as the determination of shelf life (Maszewska et al., 2018). Oxidation of edible oils can also lead to a change in its colour, taste and aroma and hence it is of utmost importance to determine the resistance of the oil to oxidation.

An automated Metrohm Rancimat apparatus was used by Latif et al. (2009) to measure the oxidative stability of the oils. The crocin kinetic competition test was used by Da Porto et al. (2012) to find out the oxidative stability of hemp seed oil extracted by supercritical fluid extraction. It was reported in his work that the highest oxidation stability of 2.16 mM Eq Vit E was obtained at 80 °C at a pressure of 300 bar.

### **2.9.5. Free Radical scavenging activity**

Production of free radicals occur when electrons are lost from the double bonds present in unsaturated triglycerides. It occurs mostly due to elevated extraction temperatures or exposure of the oils to oxygen and temperature. This would cause an acceleration of the oxidation reaction



causing the electrons to separate from double bonds resulting in the formation of free radicals which further propagates the reaction (Choe and Min, 2006). DPPH scavenging activity is a method utilized for the measurement of antioxidant capacity. It gives an idea of the amount of DPPH free radicals that are scavenged by the antioxidants present in the oil (Rezvankhah et al., 2019). It has been reported that hempseed oil contains natural antioxidants that interact with these radicals and initial oxidation products like hydroperoxides. The dominant antioxidants in hempseed oil, the tocopherols protect the oil from such oxidation reactions.

The free radical scavenging activity of vegetable oils was investigated using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate free radical (DPPH) method by Espin et al. (2000). Rezvankhah et al. (2019) also measured the antioxidant activity of his extracted hemp seed oils using the DPPH radical scavenging activity. Their work reported that higher microwave power operating at a higher temperature could extract more tocopherols in the oil, leading to more free-radical uptake. A microwave power of 450 W operating for 10 minutes provided the maximum free radical scavenging activity.

#### **2.9.6. Tocopherol**

Tocopherols are a class of organic chemical compounds which represent the Vitamin E group. They mostly consist of 4 isomers with varying functions-  $\alpha$ ,  $\beta$ ,  $\delta$  and  $\gamma$ . They are the major antioxidants present in vegetable oils. Antioxidants present in hemp seed oils are believed to stabilize the high unsaturation of the oil preventing it from becoming rancid (Small and Marcus, 2019).

The principles of microcalorimetry to determine the content of Vitamin E in vegetable oils and animal fats were studied by N.V. Sizoval (2014). This technique is based on the tocopherol

capacity to inhibit liquid phase radical oxidation reactions. HPLC analysis was performed by Kriesse et al. (2004) to assess the quantity of tocopherols present in 51 genotypes of *Cannabis sativa* L. Blade et al. (2005) and Oomah et al. (2003) also analyzed the tocopherol content of oils extracted from the hemp seeds grown in the prairie region of Canada by using normal phase HPLC. Furthermore, Oomah et al. (2002) analyzed the Manitoba grown hemp seed oil tocopherol content using HPLC method.

#### **2.9.7. Carotenoid content**

Carotenoids are considered as ubiquitous phytochemicals which plays a major role in various processes. More than 700 pigments constitute the group of carotenoids out of which the most studied ones are  $\alpha$ -carotene,  $\beta$ -carotene, lycopene and lutein (Dhan and Charu 2014).

It is believed that carotenoids play a major role in quenching singlet oxygen atoms and intercepting deleterious free radicals and reactive oxygen species inside the human system (Dhan and Charu, 2014). It has also been reported that human diet supplemented with carotenoids plays a key role in the reduction of chronic conditions related to coronary heart disease, certain types of cancers and atrophy of muscles (Sarry et al. 1994; Van Het Hof et al. 1999; Woodall et al. 1997).

It has been also shown that carotenoids tend to accumulate in the skin and tend to possess scavenging action on ROS as well as anti-inflammatory properties and as a result gained a lot of importance in the cosmetic industries due to its photoprotective properties (Stahl and Sies 2007).

Some slight modifications were incorporated to the AOAC standard methods to measure the carotenoid content of oils extracted from hemp seeds by Oomah et al. (2002). The actual carotenoid content of the hempseed oil was reported to be between 2 and 5.3 mg/100 g of oil. It was also noted that the carotenoid content increased significantly with continuous microwave heating for

six minutes.

## 2.10. Energy Calculations

Energy calculations of each of the different extraction methods is very essential for understanding the overall economic feasibility of an oil extraction processing technique. The energy consumption related to the extraction of lipids from black soldier fly biomass was studied by Feng et al. (2019). For the evaluation, the individual energy consumptions in each of the individual steps for the extraction procedures was studied which included the pre-treatment process cell rupture ( $Q_H$ ), dehydration process ( $Q_D$ ), microwave-assisted extraction ( $Q_M$ ), phase separation ( $Q_P$ ) and Solvent recovery ( $Q_R$ ).

Finally, the total energy consumption ( $Q_{Total}$ ) for lipid extraction process was calculated as the sum of the individual energy consumption as follows

$$Q_{Total} = Q_H + Q_D + Q_M + Q_P + Q_R \dots\dots\dots \text{Equation 2.1}$$

It was concluded that the microwave radiation powder, lipid extraction temperature, and water content in the raw lipid feedstock had the maximum effect on the total energy consumption. Although, any quantitative information was not available in order to understand the energy consumed fully, this study showed some general rules and characteristics that may be useful for consideration in order to understand the process.

## 2.11. Role of moisture on plant-based extraction

Moisture content (m.c.) is considered to be an essential parameter which plays a key role in extractability of oil from seeds. Orhevba et al. 2013 reported that neem seed kernels with higher moisture content yielded less oils as compared to those with a lesser moisture content. Farsie and

Singh (1995) had also reported similar results in their experiments with sunflower seeds. It was found that the optimum extraction took place at 6% moisture content, whereas it decreased as the m.c. was increased to 14%. A moisture content of more than 9% decreased the oil yield in the experiments of Sivala et al. (1991) with canola seed. Singh et al. (2019) reported that the residual oil in canola seed cake first decreased and thereafter increased as the moisture content and the heating time increased. On the other hand, the residual oil in the meal decreased with an increase in the m.c. and heating time. Moisture content and extractability of oils also depend upon the method of pre-treatment and process parameters.

## CHAPTER 3

### 3. Materials and methods

Industrial hemp hearts were procured from Northern Nutraceuticals, Saskatchewan, Canada. The plants were grown in central Saskatchewan, near Saskatoon. The harvest time for the plants was 4 months.

After 4 months, the seeds were removed from the plants and dehulled and split using dehullers of proprietary design. Around 500 g of the hemp hearts was sieved using two sieves (The W.S Tyler Company of Canada Limited) of aperture sizes 1.40 mm and 1.00 mm. A part of the whole hemp hearts was also ground which was measured to be 0.85 mm or less by a sieve. The sieved (whole and ground seeds) samples were stored in airtight zip-lock bags until the extraction experiments were conducted which was completed within two months from the time of procuring the hemp hearts. They were further crushed to obtain different size fractions (coarse to fine).

Certified ACS grade hexane (98.5%), acetone and methanol (99.8%) were purchased from Fischer Chemical, USA. 0.1 M volumetric WIJIS solution was purchased from Fluka analytical, Germany. Laboratory grade Millipore water from Milli-Q® IQ 7003/05/10/15 Water Purification System was used throughout the experiment. ACS grade phenolphthalein and reagent grade Carbon tetrachloride (99.9%) was purchased from Sigma Aldrich, USA. USP/FCC standard potassium iodide and certified ACS grade potassium hydroxide (85.8%) were obtained from Fisher Chemical, USA. Certified ACS grade starch for iodometry was purchased from Fisher Scientific, Canada. ACS certified sodium thiosulphate pentahydrate (99.5-101%) crystals were purchased from EMD chemicals, Germany.

### 3.1. Dimensional measurement:

Two sieves (CE Tyler Standard) of pore width 1.4 mm and 1.00 mm were used to initially separate the hemp seeds into two different particle sizes- 1.4 mm and larger which were designated as “whole seeds” and between 1.00 and 1.4 mm which were labeled as “medium seeds”.



**Figure 3.1**



**Figure 3.2**

**Figures 3.1, 3.2: Sieving of the hemp heart into different sizes using CE Tyler Standard Sieves**



**Figure 3.3: Whole seed hemp hearts bulk**



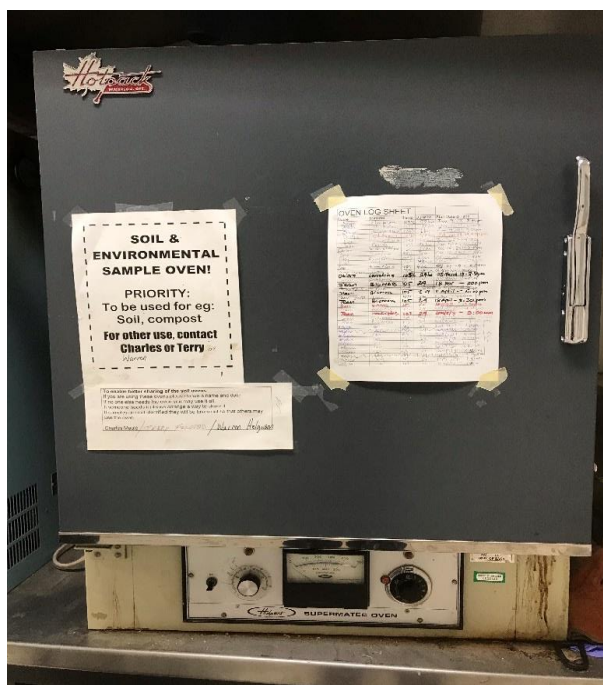
**Figure 3.4: Individual hemp hearts**

The whole seeds were further ground using a kitchen coffee grinder and sieved using CE Tyler standard sieves of pore dimension 0.85 mm. The particle size of the seeds designated throughout our experiments as “ground seeds” was measured to be 0.85 mm and less.

Throughout this thesis, the terms whole, medium and ground seeds will be used to refer to seed of the three particle sizes as mentioned above.

### **3.2. Measurement of moisture content:**

Moisture content is a critical parameter in order to understand the extraction of oils. Various researchers have reported a correlation between the moisture content of the seeds and the rate of extraction. Also, since a couple of the extraction techniques that we used in our research deal with the use of microwaves, moisture content plays an important role, since the basic principle of microwave heating depends upon the transfer of electromagnetic energy to the water molecules (which are bipolar in nature) by virtue of which their rotational energy increases. This leads to an increase in motion, whereby the intermolecular hydrogen bonds break, and the cell membranes are disintegrated. The traditional oven dry method was used to measure the initial moisture content of the seeds.



**Figure 3.5: Conventional laboratory oven set up**

5 g of both the whole and medium seeds were taken in an aluminum tray and left overnight in a Hotpack SUPREMATICS laboratory oven at a temperature of 103 °C for 24 hours. After 24 hours, the weight of the oven dried seeds was measured to be 4.732 g and 4.72 g respectively.

Moisture content was calculated by using the following formula:

$$MC = \frac{\text{Initial weight} - \text{Oven dry weight}}{\text{Oven dry weight}} \times 100 \dots \dots \dots \text{Equation 3.1}$$

The initial % of moisture (dry basis) contained by the two particle sizes are:

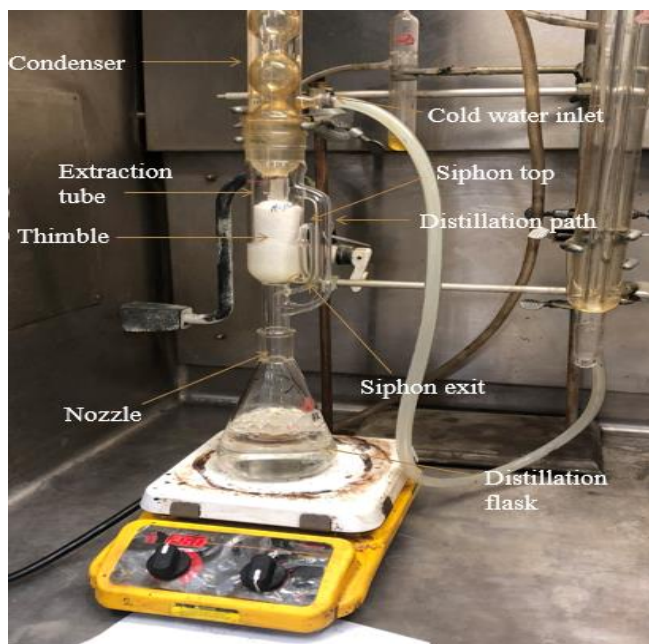
1. Particle size of 1.4 mm = 5.66
2. Particle size of 1.00 mm = 5.93



### 3.3. Extraction of oils from hemp hearts

#### 3.3.1. Soxhlet extraction:

Soxhlet extraction was carried out for each of the three particle sizes using n-hexane (ACS grade) as the solvent. Thirty-five g of 2 batches of the whole seeds was extracted with 400 ml of the solvent (n-hexane). Twenty-five g of 2 batches of both the medium and ground seeds were extracted with 400 ml of the solvent. For each run, the above-mentioned quantity of the sample was placed in a cellulose thimble which was inserted inside the Soxhlet apparatus. The apparatus was fitted to a 1000 ml round bottom flask containing the solvent. A constant temperature of around 100 °C was maintained in CIMAREC heating mantle (Thermo Fisher Scientific, Canada) during each of the runs and cold-water flow was maintained at a moderate pace. The extraction reaction was run for 4 hours for each batch of the hemp hearts. Upon completion of the extraction, the extracted samples were further dried in a laboratory oven at 100 °C to ensure the removal of traces of the residual solvent. The remaining crude residue was weighed and the % yield was calculated.



### **Figure 3.6: Conventional Soxhlet apparatus**

This mixture of oil and solvent was then transferred to a rotavapor to separate the oil from the solvent. For our experimental purposes, a BUCHI Waterbath B-480 (U.S.A.) was used to maintain a temperature of around 70 °C. The boiling point of hexane is 65 °C. Hence, the temperature of the water bath was maintained at around 70 °C, so that the solvent evaporated and passed to the condenser. A rotary vacuum evaporator (Rotavapor®, Buchii, Farmingdale, NY) maintained the rotation of the conical flask where our mixture was present at a constant speed of 2.5. BUCHI Vacuum pump V-700 (U.S.A.) maintained a vacuum pressure of around 360 mbar for the entire separation process to take place. A VWR chiller maintained the temperature of the condenser around -3 °C to -4 °C. The entire purification process took around 30-45 minutes. Thereafter, the oil was transferred to a clean glass jar and weighed to cross check the yield %.

#### **3.3.2. Microwave Pretreated Soxhlet extraction:**

A Panasonic DIMENSION 4<sup>th</sup>Genius microwave (U.S.A.) was used to pre-heat the hemp hearts. According to the reader's manual, Table 3.1 denoted the relationship between the micropower readings and the output power of the microwave oven.

**Table 3.1: Microwave power levels and their indices**

<b>Micropower (factory settings)</b>	<b>Power (Watt)</b>
1	90
2	180
3	270
4	360
5	450
6	541
7	630
8	720
9	810
10	900

From the Table 3.1, we concluded that since the maximum output power of the microwave is 900 W, a micropower of 10 in the machine corresponds to a power output of 900 W. Accordingly, a micropower of 5 corresponds to a power output of 450 W and a micropower of 7 corresponds to a power output of 630 W.

We alternated the time and power based on previous literature to find the optimum heating power and time that might produce maximum oil extraction. The optimization results along with the outcome of the samples are presented in Figure 3.7.



**Figure 3.7: Different power and times used to treat the hemp heart samples**

P indicates output micropower of the microwave oven (in Watts) and T indicates the time in minutes (min).

**Table 3.2: Comparison between different combinations of Microwave power and time**

Micropower	Time (min)	Preference
5	5	Preferred
10	5	Not preferred
1	5	Not preferred
7	5	Preferred
10	7	Not preferred
7	3	Preferred
10	3	Not preferred
5	3	Not preferred

Based on previous literatures, we concluded that the time and power that might result in the maximum extraction of oil is T-5 and P-5 i.e. a power of 450 W for a time of 5 minutes.

For the experiment, 35 g of 2 batches of the sample of whole seeds and 25 g of 2 batches of the sample of medium as well as the ground seeds were distributed into aluminum containers

and pretreated with a micro-power of 5 (450 W) for 5 minutes. Thereafter, Soxhlet extraction was performed as previously described in Section 3.3.1. and the oil was purified using a rotary vacuum evaporator (Rotavapor®, Buchii, Farmingdale, NY). The collected oil samples were properly labelled and stored inside a refrigerator (VWR International, Edmonton, AB) at a temperature of -4 °C for further analysis.

### **3.3.3. Ultrasound Assisted Soxhlet extraction**

For the purpose of the experiment, a GmbH UP400S Ultrasonic processor (Hielscher Ultrasonics, Mount Holly, NJ) was used to sonicate the seeds. 35 g of the whole seed samples was used for the purpose. The ultrasonic probe used for the process was H7. The working frequency was unaltered and maintained at the working frequency of the device that is 24 kHz. The pulse-pulse mode factor was maintained at 100% (1 of the machine). The power input was maintained at 200 W (50%) and the run time was 12 minutes.

After that, the samples were transferred to a Soxhlet apparatus and traditional Soxhlet extraction was carried out with 400 ml of n-hexane for 4 hours at a temperature of 65 °C to 70 °C. After that, the oil was separated from the solvent-oil mixture using a rotary vacuum evaporator as above and was stored at -4 °C for further analysis.

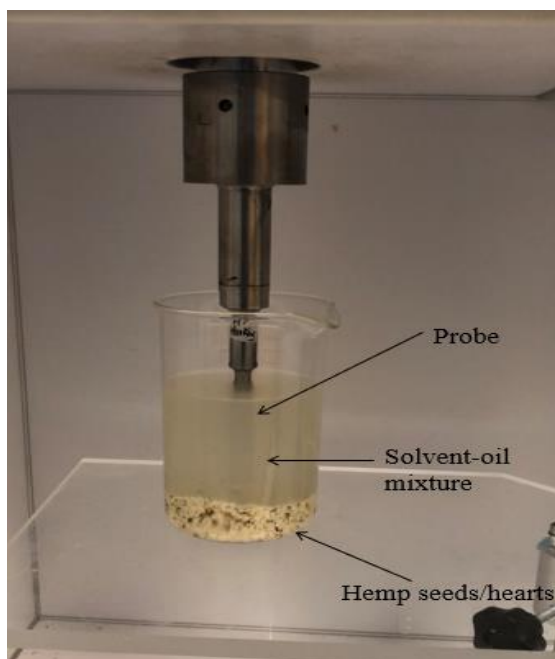
### **3.3.4. Ultrasound extraction of oils from hemp seeds**

For the purpose of Ultrasound extraction, a GmbH UP400S Ultrasonic processor (Hielscher Ultrasonics, Teltow, Germany) fitted with a H7 probe was used to sonicate the seeds immersed in n-hexane. The ratio of solid to solvent was maintained at 1:10 (w/v) based on previous literatures. 25 g of both the whole and ground hemp heart samples were mixed with 250 ml of the solvent and introduced inside the ultrasonic processor. The experimental conditions used are tabulated in Table 3.3.

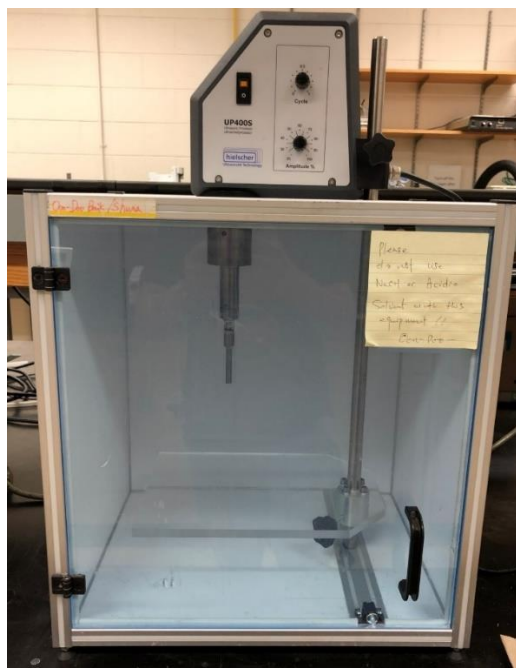
**Table 3.3: Conditions used for ultrasound treatment of hemp hearts**

<b>Experiment no.</b>	<b>Sample weight (g)</b>	<b>Sample type</b>	<b>Solvent (ml)</b>	<b>Ultrasound Power (Watts)</b>	<b>Time (min)</b>	<b>Pulse</b>
1	25	whole	250	200	30	0.5
2	25	ground	250	200	30	0.5
3	25	whole	250	200	60	0.5
4	25	ground	250	200	60	0.5
5	25	whole	250	200	10	0.5
6	25	ground	250	200	10	0.5
7	25	ground	250	20	60	0.5
8	25	ground	250	80	35	0.5
9	25	ground	250	130	10	0.5
10	25	ground	250	130	60	0.5

For all the experiments, the operating frequency of the device was maintained at 24 kHz. The solvent-oil mixture was separated from the extracted hemp hearts by vacuum filtration using a Buchner funnel. The solvent laden seeds were kept in the oven for 3 hours before they were weighed and stored in plastic containers. The oil was separated from the solvent by using a Rotavapor and stored in the refrigerator at -4 °C for further analysis.

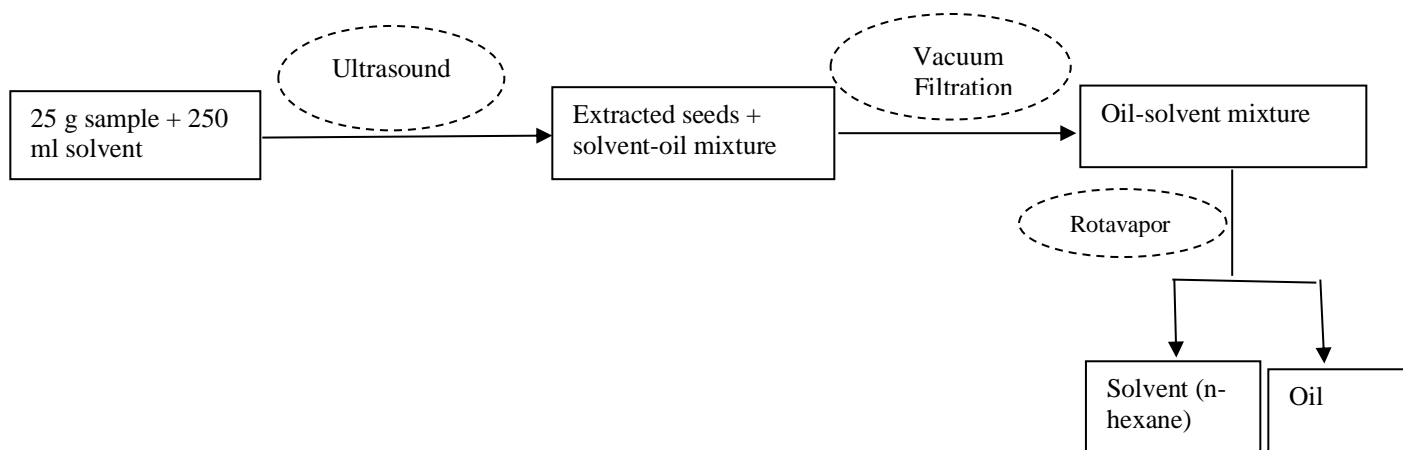


**Figure 3.8: Ultrasound treatment of hemp hearts**



**Figure 3.9: Ultrasound apparatus**

The experimental procedure is represented in the form of a flow-chart in Figure 3.10.



**Figure 3.10: Flow chart showing the extraction of oil using ultrasound extraction**

### 3.3.5. Microwave extraction

An Explorer Hybrid<sup>12</sup> microwave extractor (CEM Discover, U.S.A.) was used for the extraction of oils from the hemp heart samples.

The Microwave conditions that we have used in our experiment is tabulated in Table 3.4.



**Figure 3.11: Laboratory Microwave extractor Setup**

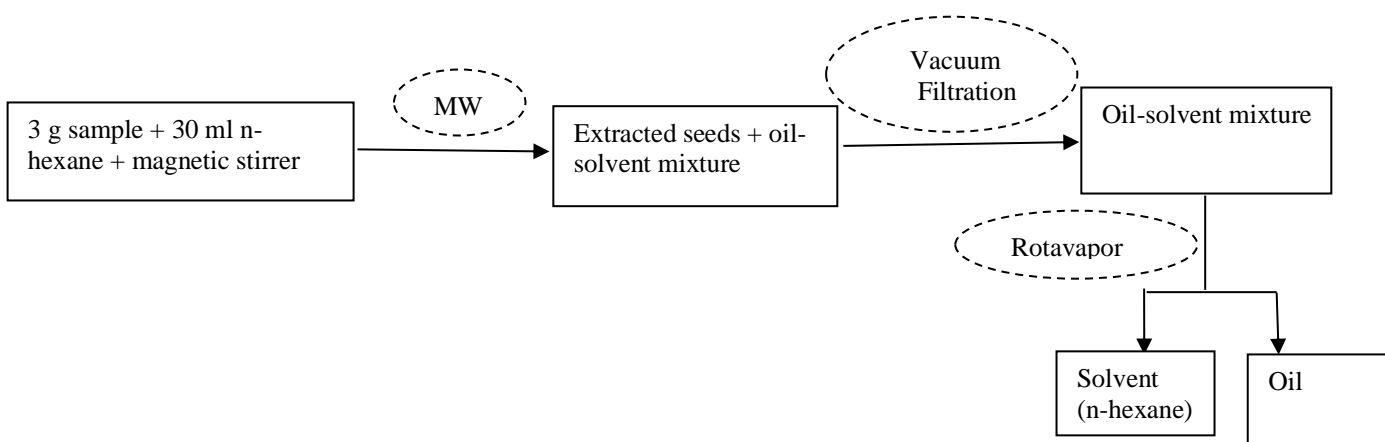
**Table 3.4: Experimental conditions for Microwave Extraction**

Experiment no.	Time (min)	Temperature (°C)
Experiment 1 (Batch 1, Batch 2, Batch 3)	15	65
Experiment 2 (Batch 1, Batch 2, Batch 3)	20	70
Experiment 3 (Batch 1, Batch 2, Batch 3)	10	70
Experiment 4 (Batch 1, Batch 2, Batch 3)	30	65



For each of the experimental conditions, three batches of hemp hearts, each containing 3 g of the sample was dissolved in 30 ml of the solvent n-hexane, taken in an extraction tube and placed inside the microwave extractor. A magnetic stirrer was added to the tubes for homogenous mixing. The software Synergy was used to input the above conditions in the computer and the experiment was run. After each experiment, the mixture of oils and extracted seeds were emptied in a glass container. Vacuum filtration was performed to separate the oil-solvent mixture from the extracted seeds. Thereafter, the mixture was purified, and pure oil was collected using a Rotavapor. The extracted oils were stored in glass vials in the refrigerator at 4°C for further analysis.

The experimental procedure is represented as a flowchart in Figure 3.12.



**Figure 3.12: Flow-chart representing the extraction of oil using a Microwave extractor**

### 3.3.6. Cold pressing

#### 3.3.6.1. Material:

2 kg of the dehulled hemp heart sample was used for the purpose of cold pressing.

#### 3.3.6.2. Moisture content:

For the process of cold pressing, a moisture content of around 8-10% was desired. The initial moisture content of the seeds was 5.66 % and hence tempering was performed to bring the moisture

content up to the desired value.

### 3.3.6.3. Tempering of the seeds:

Around 500 g of the hemp hearts was taken in 4 air-tight jars. The following calculations were performed to calculate the amount of water to be added to bring the moisture content up to 8-9 %.

$$\text{Moisture content} = \frac{\text{Mass of water}}{\text{Mass of dry solid (MS)}} \dots\dots\dots \text{Equation 3.2}$$

$$\frac{5.66}{100} = \frac{\text{Mass of water (MW1)}}{\text{Total mass(MTot)-MW1}}$$

$$\frac{5.66}{100} = \frac{\text{MW1}}{500-\text{MW1}}$$

$$0.0566 \times (500-\text{MW1}) = \text{MW1}$$

$$28.3 - 0.0566\text{MW1} = \text{MW1}$$

$$1.0566\text{MW1} = 28.3$$

$$\text{MW1} = 26.784 \dots\dots\dots \text{Equation 3.3}$$

$$\text{Or MS} = 500 - 26.784 = 473.216 \dots\dots\dots \text{Equation 3.4}$$

The target was to achieve a moisture content of 8.5 %

$$\text{Therefore, } 0.085 = \frac{\text{MW2}}{473.216}$$

$$\text{Or, MW2} = 40.223 \text{ g} \dots\dots\dots \text{Equation 3.5}$$

From Equation 5, it was understood that 26.784 g water was already present in 500 g sample.

Therefore, to make the water content rise up to 40.22 g, the amount of water to be added to the hemp heart samples was (40.22-26.78) g or 13.43 g.

Thus, 13.44 g of water was added into each of the jars and shaken vigorously so that the water was evenly absorbed. The jars were then kept inside the cold room at a temperature of  $4 \pm 0.5$  °C for 48 hours. The jars were manually shaken every hour initially (for the first 6 hours) and thereafter at an interval of 8-10 hours for uniform absorption of the water by the seeds. After 48 hours, the

seeds were taken out from the cold room. The moisture content was measured by the oven dry method and found out to be 9.5%.

#### **3.3.6.4. Instrumentation and optimization**

An Oekotec screw-press (MONFORTIS IBG, Nordrhein-westfalen, Germany) was used for the purpose of cold pressing of oil from the hemp heart samples. The hemp hearts were mixed with some portion of the hulls for the purpose of the ease of extraction, otherwise, the screw would press the seeds into a paste and that would not result in maximum extraction of the oils.



**Figure 3.13: Industrial Screw press**

Three trials were performed and for each trial 600 g of the hemp heart sample was mixed with 8.25% or 49.5 g hulls and pressed using a die of 0.6 cm diameter. The motor of the press was maintained at a frequency of 24 Hz (40% of the transmitter) using an INVEOR transmitter.

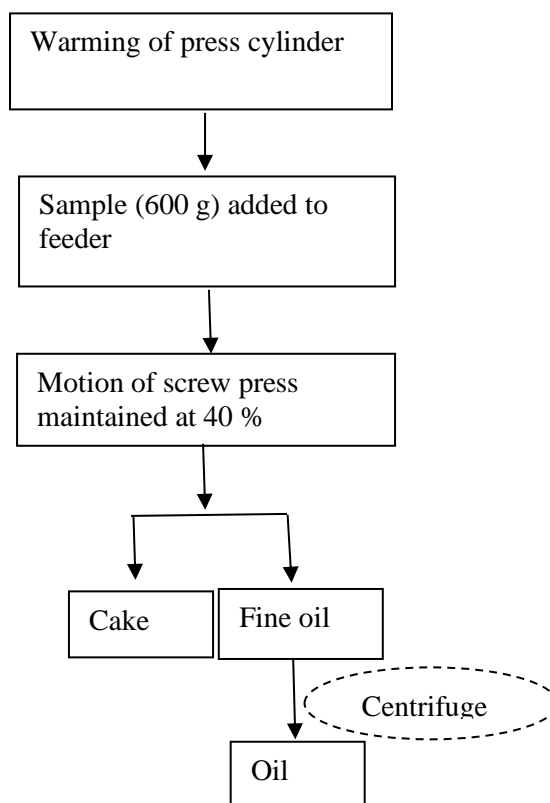
From previous experiments using 2%, 4%, 6% hulls, it was found out that the cake coming out of the press cylinder still contained a considerable amount of the oil and had to be run again through

the screw press. An added hull % of 8 and 10 achieved maximum extraction of oils from the hearts without showing much difference in the extraction efficiency. Hence, we chose the hull percentage of around 8.25%. Moreover, from past experiments, it was found that a die of diameter 0.8 cm resulted in a higher residual oil content in the pressed cake. Also, a die of diameter smaller than 0.6 cm resulted in non-uniform pressing due to frictional forces inside the cylinder and thus resulted in contamination of the extracted oils with the cake and in turn led to errors in the final result. A die of diameter 0.6 cm was found out to be optimal for obtaining maximum extraction of oils from the hemp hearts and hence was used for the pressing trials.

#### **3.3.6.5. Experimental procedure:**

At the start, the pressing cylinder was heated to a temperature of around 60 °C to ensure uniform pressing. Thereafter, around 600 g of the sample was added to the feeder which passed to the pressing chamber. The oil expelled out of cage housing (cage openings of 1.5 mm diameter) in the press cylinder and was collected in a clean tub. The press cake was also collected and weighed for further analysis. The oil was further purified by using a J6-M1 centrifuge (BECKMAN COULTER, Brea, CA) operating at 4200 R.P.M for 10 minutes at a temperature of 18 °C.

The experimental procedure is represented in the form of a flow chart in Figure 3.14.



**Figure 3.14: Flow chart showing the mechanism of cold pressing**

In order to understand the amount of oil actually produced from the cold pressed hemp hearts, the Swedish tube method was performed on the hemp hulls to find the quantity of oil contributed by them. Around 2.22 g of the hemp hulls was taken for the purpose of measuring their oil content. The residual oil in the press cake was determined and reported by the Analytical Service Laboratory at the KeyLeaf Life Sciences (Saskatoon, SK) using the Swedish Tube method of analysis (Troëng, 1955). Approximately 3.15 g of the initial hemp hearts was also analyzed for their initial oil content using the Swedish tube method.

### **3.4. Analysis of the compounds from extracted oils:**

#### **3.4.1. Presence of different chemical components**

##### **3.4.1.1. Gas Chromatography Mass Spectrometry**

For the analysis of compounds, a Trace 1310 Gas Chromatography system (Thermo Fisher

Scientific, Edmonton, AB) been used which was equipped with a Thermo Scientific ISQ LT Single Quadrupole Mass Spectrometer.



**Figure 3.15: Laboratory GC-MS Apparatus**

The temperature of the source was set at 250 °C and the helium flow rate was 1.2 mL/min. One  $\mu\text{L}$  of each sample was injected at 250 °C with a split ratio of 50:1 and split flow of 60 mL/min. The oven temperature was initially set to 40 °C with a hold time of 1 min, then increased to 150 °C at 5°C/min and finally, increased to 320 °C at 10 °C/min and held constant for 5 min. The hemp heart oil samples were dissolved in acetone for the preparation of test samples for GC-MS analysis. The mass spectral data for the bio-crude samples were acquired from 50 to 650 m/z. The peaks were identified after comparison against the standard NIST (National Institute of Standards and Technology) library using Chromeleon™ 7.2 (Thermo Fisher Scientific, Edmonton, AB) Chromatography Data System (CDS) software.

#### **3.4.1.2. Nuclear Magnetic Resonance Imaging**

The extracted oils from each of the extraction techniques (SE, MWPS, US and CP) were analyzed

using  $^1\text{H}$  NMR spectroscopy. A 500 MHz automatic Avance NMR (Bruker, Hamilton, ON) equipped with a TX1 and BBO probe was used for the analysis.



**Figure 3.16: Laboratory NMR Apparatus**

Around 0.2 ml (1-2 drops) of the sample oil (reaction samples) was pipetted into NMR tubes (5 mm; Norell Standard Series, Sigma-Aldrich, USA) and then deuterated chloroform ( $\text{CDCl}_3$ ; Sigma Aldrich, Oakville, ON) was added to dissolve the low polarity esterified reaction mixtures. Where the reaction mixture was rich in partial esters, deuterated methanol ( $\text{MeOD}$ ) (Sigma-Aldrich, USA) was used instead of deuterated chloroform. The unprocessed free induction decay (fid) data were converted to frequency domain by Fourier transform (ACD Labs version 11). Manual baseline correction and integration was applied using the Topspin 4.0.7 software package (Bruker, Bremen, Germany).

### **3.4.2. Antioxidants**

#### **3.4.2.1. Tocopherol**

The tocopherols were measured and identified from the GC-MS data after comparison against the standard NIST (National Institute of Standards and Technology) library using Chromeleon<sup>TM</sup> 7.2

Chromatography Data System (CDS) software.

#### **3.4.2.2. $\beta$ -carotene**

The quantity of  $\beta$ -carotene was measured in the sample by using the British Standard methods of Analysis BS 684-2.20 (1977) using a Shimadzu mini 1240 UV-VIS spectrophotometer (Shimadzu corporation, Houston, TX, U.S.A.). For the experiment, 0.25 g of the oil was dissolved in cyclohexane and made upto 25 ml in a volumetric flask. Around 3.5 ml of the solution was taken in a quartz cuvette and the absorption was measured at 450 nm. The amount of  $\beta$ -carotene was measured using the following formula (Tesfye et al. 2017).

$$\beta\text{-carotene} = \frac{V \times 383 \times (A_s - A_b)}{100 \times W} \dots\dots\dots \text{Equation 3.6}$$

where:

V= Volume of the solution used for analysis

$A_s - A_b$  = Absorbance of the sample – Absorbance of the blank

W= Weight of the sample in gram and

383= Molar Extinction co-efficient for carotenoids.

### **3.5. Measurement of Physical properties:**

#### **3.5.1. Color of oil:**

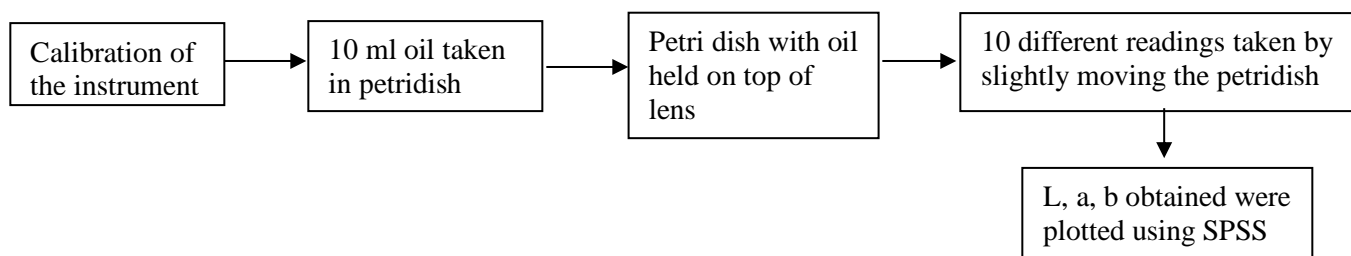
For the purpose of measurement of color, a Folio Kitchener 51-748-4612 hand-held spectrophotometer (Konica Minolta, Saskatoon, SK) was used.





**Figure 3.17: Laboratory hand-held spectrophotometer**

The color of the oil obtained by each extraction technique was measured. The experimental procedure is shown in the form of a flow chart in Figure 3.16.



**Figure 3.16: Measurement of color by Konica Minolta hand-held spectrophotometer**

The instrument was calibrated firstly against the surrounding laboratory environment and then against the standard white color present in the device cap. After calibration, an aliquot of 20 ml sample was taken in a clean petridish and placed on top of the lens and the reading was noted. 10 readings from each oil sample was taken for maintenance of uniformity and consistency and this was achieved by slightly moving the petridish on top of the lens so that ten different positions of

the sample got exposed to the lens. The results were interpreted by Color Data Software CM-S100w SpectraMagic™ NX (Konica Minolta, Saskatoon, SK) Ver. 2.8 which gave the values in terms of L, a and b. The L, a and b values were further fed to SPSS software for better understanding of the difference in the values of L, a and b using graphical representations.

### 3.5.2. Viscosity:

The viscosity of the samples was measured using a DV-1+ Digital Viscometer (BROOKFIELD, MA, USA). The viscometer drove a CPV 407 spindle which was connected to a rotary transducer by means of a spring.



**Figure 3.18: Laboratory Digital Viscometer**

#### 3.5.2.1. Calibration of device

Before starting the experiment, the device was calibrated using an oil of known viscosity (96 cP).

An aliquot of  $0.5 \pm 0.1$  ml of the standard oil was transferred into the viscometer cup. The cup was fitted to the device and the micrometer adjustment ring was steadily rotated until the viscosity displayed by the device was 96 cP.

### 3.5.2.2. Newton's Law of viscosity test

Firstly, the oil samples were tested to see if they followed Newton's laws of viscosity i.e. if they were Newtonian or non-Newtonian in nature.

For the purpose, 0.5 ml of the ultrasound extracted oil (any one oil sample could have been used) was distributed into the viscometer cup and fixed with the device. Throughout the experiment, the temperature of the water bath was maintained at 25 °C and the shear rate varied between 1.5 to 12 RPM to check if there was a huge difference in the measured viscosity. The conditions used are shown in Table 3.5.

**Table 3.5: Variation in the % Deflection and Viscosity with respect to the RPM**

<b>RPM</b>	<b>% Deflection</b>	<b>Viscosity (cP)</b>
0.6	4.9	25.5
1.5	13.7	28.2
3	27.9	28.7
6	59.4	30.3
12	Error	Error

### 3.5.2.3. Measurement of Viscosity

The viscosity of the oils was measured at 25 °C and 40°C at an RPM of 3 and 6 respectively for each oil sample. For measurement, 0.5 ml of each sample was uniformly spread in the viscometer cup and fixed to the device which was in contact with the spindle. The viscous drag exerted by the fluid on the spindle was measured by the deflection of the spring connected to the spindle. A rotary transducer connected to the spring gave the viscosity and % deflection readings.

## 3.6. Measurement of Chemical properties

### 3.6.1. Iodine value by <sup>1</sup>H NMR Spectroscopy

Iodine value is a measure of the unsaturation present in fats and oils and is expressed in terms of the number of grams of iodine absorbed per 100 gram of the oil. Iodine value of the extracted oils was calculated according to Popescu et al. 2015 by using the integrated values of the peaks obtained from <sup>1</sup>H NMR. The higher the iodine value, the higher is the degree of unsaturation.

The values obtained after integrating the peaks using Topspin 4.0.7 were substituted into an empirical formula for the calculation of Iodine value which is as follows (Popescu et al. 2015; Vigli et al. 2003; Mannina et al. 2003):

$$\text{Iodine value} = \frac{\frac{(10)}{2}}{[(2)+(1)]/3} \times 86 \dots\dots\dots \text{Equation 3.7}$$

Where 86 represents the IV of Oleic acid.

### 3.6.2. Acid value

Acid value is defined as the number of mg of KOH which is necessary to neutralize the FA present in 1 g of the sample. It is a measure of the amount of FFA that is present in the oil. The acid value of the hempseed oil samples was measured according to AOCS official method Cd 3d-63 with a slight modification. In our experiments, we used ACS grade 95 % EtOH instead of a mixture of toluene and isopropyl alcohol. The experiments were performed for each of the oil samples and one for determining the blank. Around 60 ml of ethanol was taken in a 100 ml glass beaker and a drop of phenolphthalein solution was added. 0.1 N KOH was added dropwise to the ethanol so that it turned into a faint but permanent pink color. This solution was saved for later use. An aliquot of 0.5 g oil was taken in a clean Erlenmeyer flask to which 10 ml of the previously prepared ethanol solution was added and kept on a hot plate until the turbidity disappeared. Thereafter, the solution was titrated against 0.1 N KOH. The end point was achieved when the color of the colorless solution was changed to the first permanent faint pink color (as was observed previously in the

case of EtOH) and lasted for at least 30 seconds.

The following formula was used to find the Acid value:

$$\text{Acid value} = \frac{(A-B) \times N \times 36.1}{\text{Weight of the sample (gm)}} \dots\dots\dots \text{Equation 3.8}$$

Where:

A= Volume (ml) of the standard alkali used in the titration

B= Volume (ml) of standard alkali used in titrating the blank and

N= Normality of standard alkali.

### **3.6.3. Oxidative stability:**

The term oxidative stability determines the resistance of the oils and fats to oxidation. The oxidative stability of the extracted oils was determined by an accelerated oxidation test (Rancimat method) carried out at 110°C according to EN 14112 standard procedure. For the purpose of the experiment, a Metrohm 743 Rancimat (Metrohm AG, Switzerland) was used to conduct the stability tests. 50 ml Millipore water was taken in the measuring vessel. The conductivity cell with its electrical connections were incorporated in the measuring vessel cover and immersed in the measuring solution. Around 3 g of each oil sample was measured and transferred to the reaction vessel. A glass rod was inserted inside the vessel which touched the oil and the reaction vessel was covered using an airtight cap. The whole set-up was connected to the conductivity cell and the temperature was entered in the software StabNet™ (Metrohm, AG, Switzerland) which gave us the induction times after the completion of the experiment.

## **CHAPTER 4**

## 4. Results and Discussion

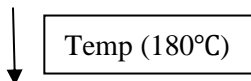
Hemp seed oils were obtained by different extraction techniques like the conventional soxhlet extraction (SE), microwave pretreated soxhlet extraction (MWPS), microwave extraction (MW), ultrasound extraction (US) and cold pressing (CP). A comparison of the extraction yields, fatty acid and antioxidant profile as well as physical and chemical properties of the extracted oils have been discussed in this section.

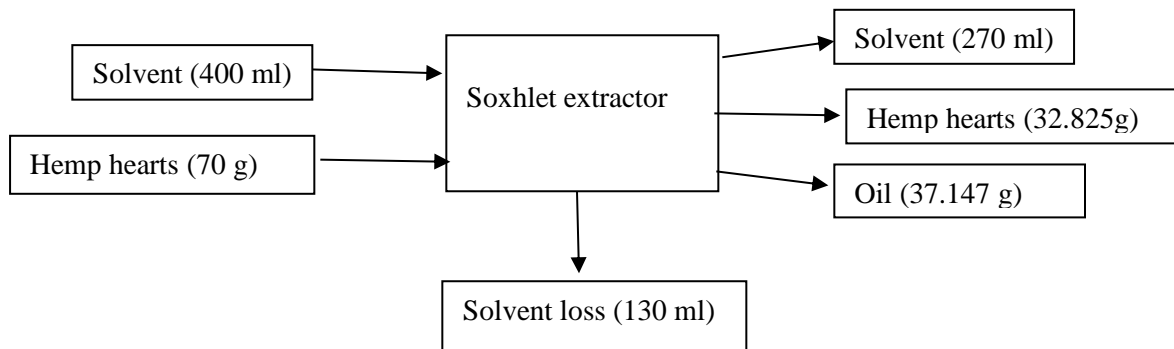
For each of the extraction experiments, except the cold pressing, two replicates have been performed i.e. each experiment was performed twice for each of the extraction conditions that was used. For cold pressing, three replicates were performed for the experiment.

For experiments on physical and chemical properties determination, two replicates have been performed for each of the experiments, except for the determination of colour. For colour determination, 10 repeated tests have been performed (averages were calculated) on each of the samples in order to achieve better results.

### 4.1. Mass Balance

The reaction taking place during Soxhlet extraction is diagrammatically represented as an example for better understanding of the mass balance that is taking place during the extraction mechanisms.





**Figure 4.1: Schematic diagram of Mass Balance for Soxhlet Extraction**

From the principles of Mass Balance, we know that,

Total Input Weight = Total Output Weight + Solvent recovered + Solvent lost .....Equation 4.1

or, {Total weight of the Solvent + Hemp heart samples} = {Total weight of solvent recovered + extracted seeds + oil + solvent absorbed by the seeds + solvent absorbed by the thimble and cotton plug + handling errors}.....Equation 4.2

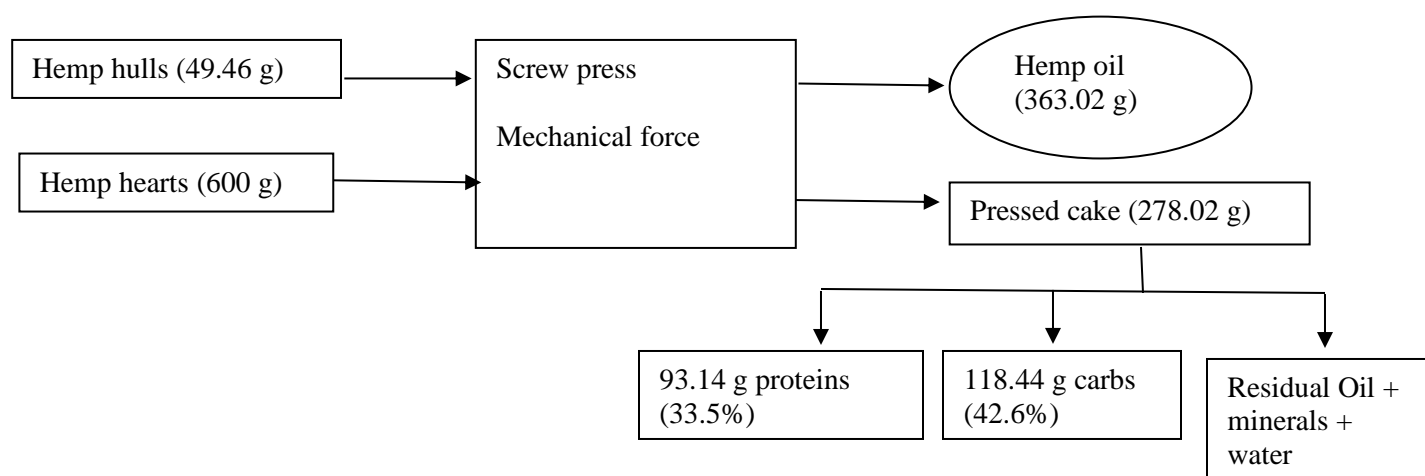
Putting the values from one of our Soxhlet extractions in the equation,

400 ml + 70g = 270ml + 32.825 g + 37.147g + 50ml + solvent absorbed by the thimble and cotton plug + handling errors.....Equation 4.3

Or, solvent absorbed by the thimble and cotton plug + handling errors = (400-320 ) ml = 80 ml  
.....Equation 4.4

Since the condenser was closed (closed Soxhlet extraction system), there was no solvent lost in the environment and hence the loss of solvent can be considered 0.

The mass balance taking place during cold pressing of the oils is also diagrammatically represented in Figure 4.2



**Figure 4.2: Schematic Mass Balance diagram for cold pressing of oils using a screw press**

According to the principle of mass balance,

$$\text{Total input mass} = \text{Total output mass} + \text{loss} \dots \dots \dots \text{Equation 4.5}$$

$$\text{or, Total weight of hemp hearts} + \text{Total weight of hemp hulls} = \text{Total weight of oil obtained} + \text{total weight of pressed cake} + \text{Loss} \dots \dots \dots \text{Equation 4.6}$$

In this example, the results obtained from one of the three trials is illustrated

$$\text{or, } 600 \text{ g} + 49.46 \text{ g} = 363.02 \text{ g} + 278.02 \text{ g} + \text{Loss} \dots \dots \dots \text{Equation 4.7}$$

$$\text{Therefore, Loss} = 8.42 \text{ g} \dots \dots \dots \text{Equation 4.8}$$

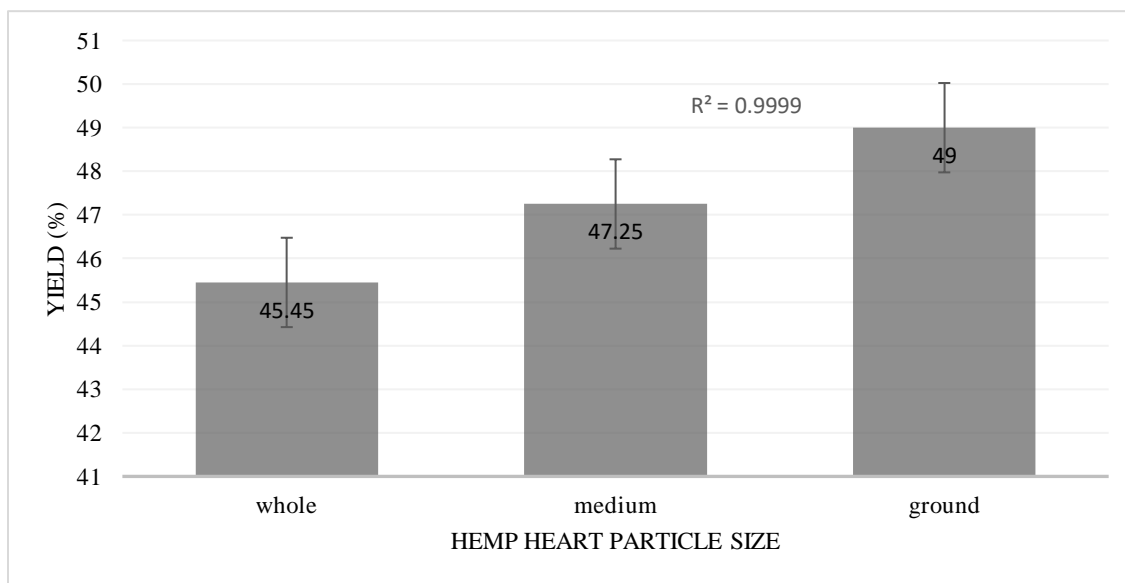
This loss can be attributed to the amount of cake lost in the expeller during the pressing mechanism.

#### 4.2. Effect of particle size on oil yield

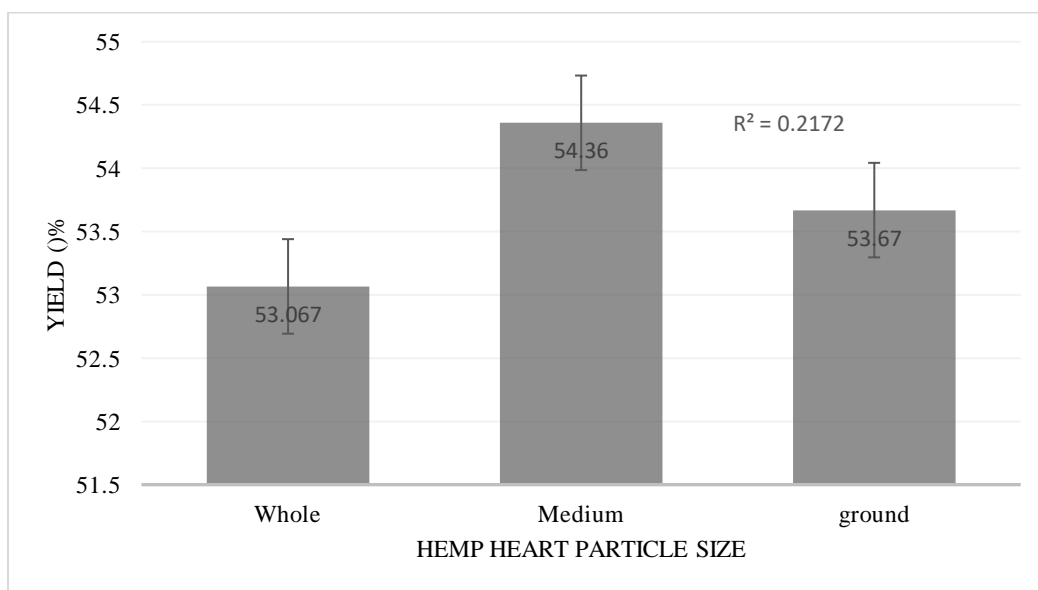
Particle size is an important parameter which should be considered during extraction studies of oils. In our study, we performed both Soxhlet extraction (SE) and Microwave-pretreated Soxhlet extraction (MWPS) using three different particle sizes- whole seeds (1.4 mm and greater), medium



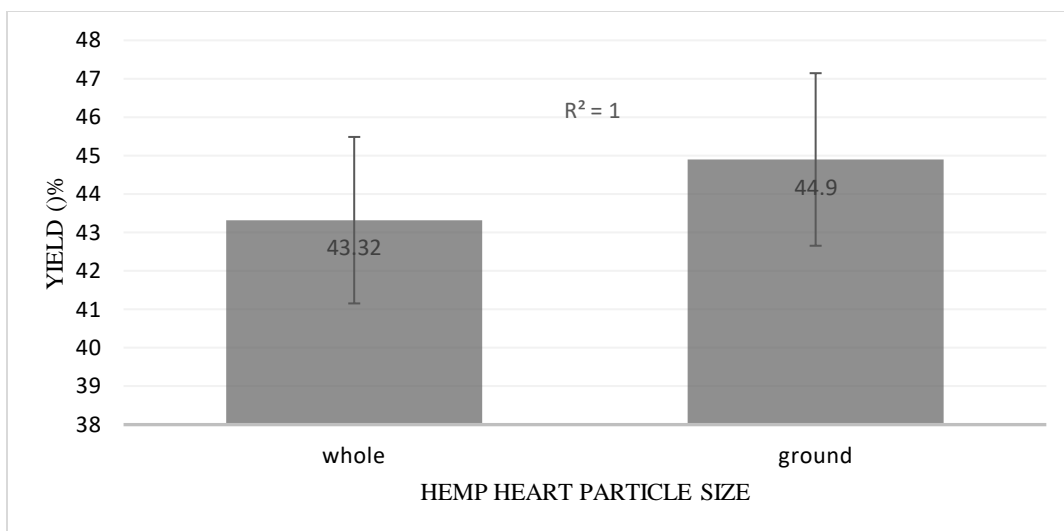
seeds (1.00 mm – 1.4 mm) and ground seeds (0.85 mm and less) as well as US extraction using whole and ground seeds. The results obtained are represented graphically in Figures 4.3, 4.4, 4.5 and 4.6.



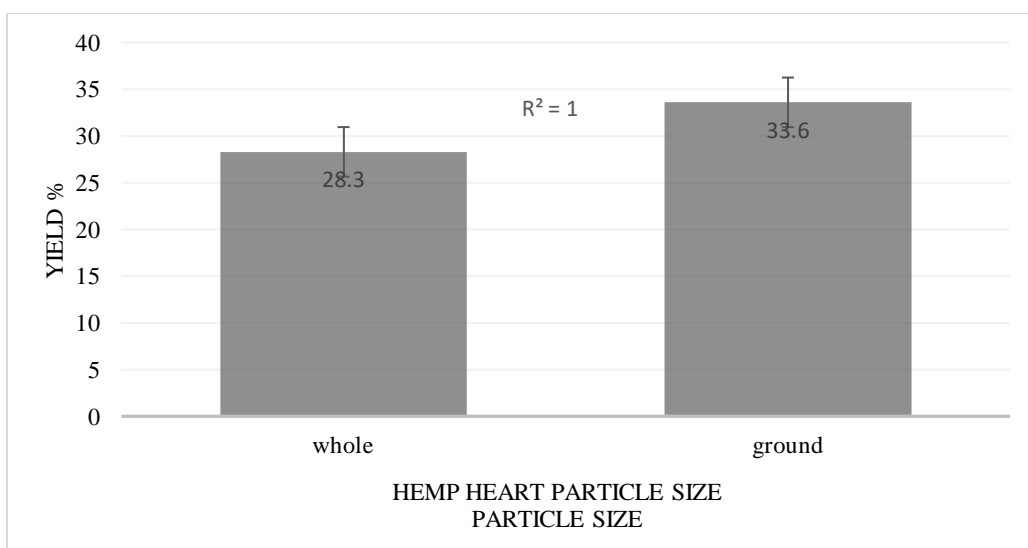
**Figure 4.3: Effect of particle size on the yield of oil for Soxhlet extraction (n=2)**



**Figure 4.4: Effect of particle size on the yield of oil for MW pretreated Soxhlet extraction (n=2)**



**Figure 4.5: Effect of particle size on the yield (%) for US power of 200 W and time of 60 min (n=2)**



**Figure 4.6: Effect of particle size on the yield (%) for US power of 200 W and time of 10 min (n=2)**

For all three extraction techniques, the oil yield increased slightly with a decrease in the particle size. For Soxhlet extraction, a maximum yield of 49% was observed for the ground seeds whereas an yield of 45.45 % was observed for the whole seeds. For ultrasound extraction, yields of 44.9 % and 33.6 % were obtained for the ground seeds in comparison to yields of 43.32 % and 28.3 % for whole seeds for ultrasound times of 60 minutes and 10 minutes respectively. This can be attributed

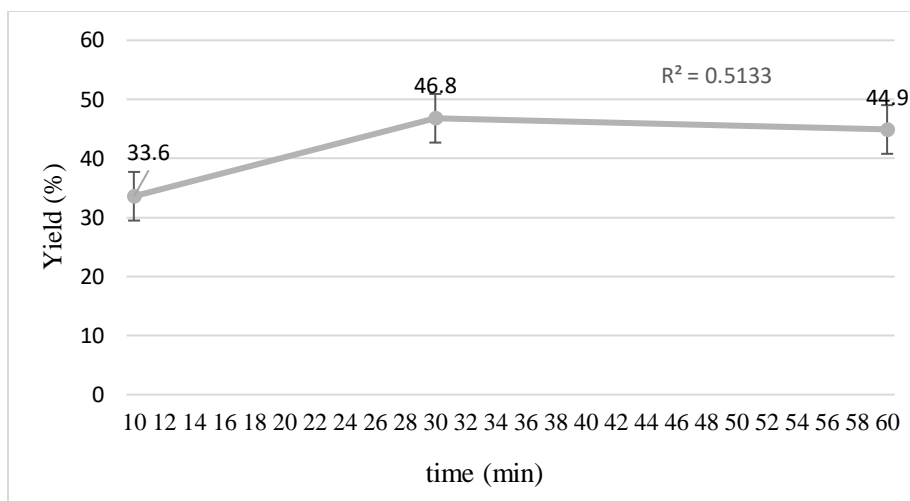
to the fact that a smaller seed size will enhance the contact surface area between the solvent and the seed surface (Reverchon 1996) and thus help in better penetration of the solvent inside the seeds leading to more extraction. In case of MW pretreated Soxhlet extraction, the maximum yield (54.36 %) was reported for medium seeds, followed by the ground seeds (53.67 %). Thus, it can be noted that further grinding to <0.85 mm did not significantly change the extraction yield. These results also go in accordance with the study of Yunus et al. (2012) who also showed that a better yield of oil is obtained from seeds having a lesser particle size.

#### **4.3. Effect of extraction variables on Ultrasound extraction of Hempseed oil**

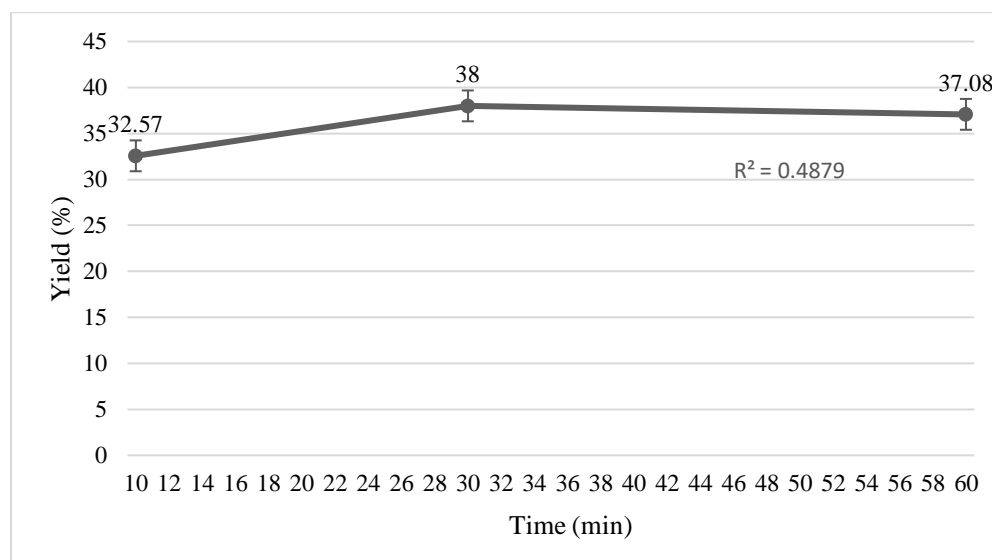
In order to study the effects of the different extraction parameters, the solvent to solid ratio was maintained at 10:1 (Lin et al. 2010) and the pulse-pulse ratio of the ultrasound device was maintained at 0.5 i.e. the acting on-off ratio was kept at 1:1 (Lin et al. 2010). The pulse-pulse ratio is the amount of time (per second) that the ultrasound pulse is generated during one cycle. A pulse-pulse ratio of 0.5 indicates that for every cycle, the ultrasound waves were generated for 0.5 seconds and were not generated for the remaining 0.5 seconds. The ultrasound power and time were adjusted in order to find the best combination of parameters for maximum extraction.

##### **4.3.1. Effect of Ultrasound exposure time on oil yield**

For the purpose of this study, the Ultrasound power was maintained at 200 W and 135 W and the hemp hearts were exposed to ultrasound for 10, 30 and 60 minutes for each of the power settings. From the study, it was noted that the yield of oil was affected by the ultrasound power. For ultrasound power of 200 W, the yield increased from 33.6 % in 10 minutes to 46.8 % in 30 minutes and then decreased to 44.9 % when exposed for 60 minutes. The same trend can be observed in case of an ultrasound power of 135 W, where the yield increased from 32.5 % to 38 % and then dropped down to 37.08 % for ultrasound exposure times of 10, 30 and 60 minutes respectively.



**Figure 4.7: Change in yield percentages with respect to the time for US power of 200 W (n=2)**



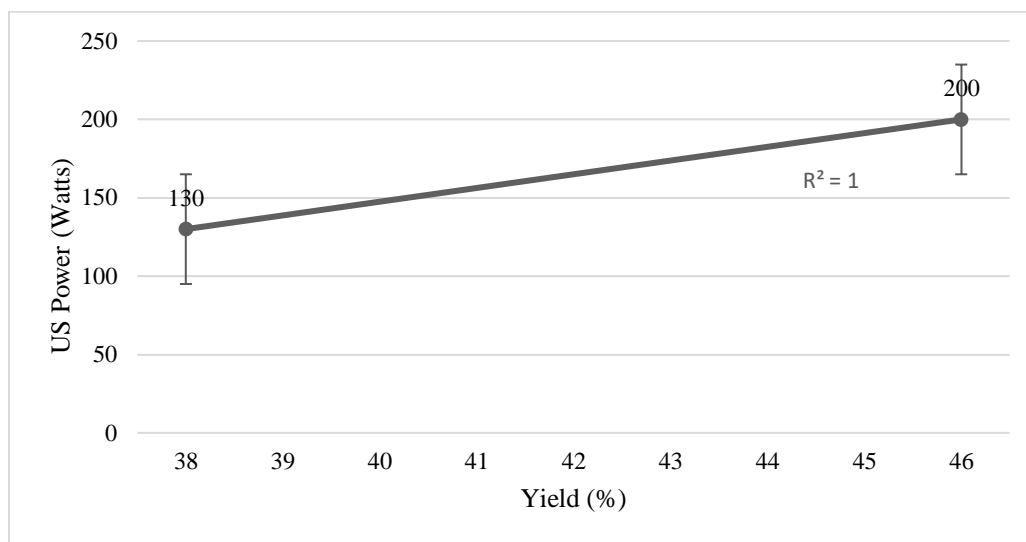
**Figure 4.8: Change in yield percentages with respect to the time for US power of 130 W (n=2)**

These results indicated that the ultrasound is more effective in the first half-an-hour action period. This may be attributed to the fact that due to increased time periods; ultrasound waves can disrupt more hemp heart cell walls leading to more penetration of the solvent inside the cells and in turn causing more extraction of oil. Again, the slight decrease in the extraction yield upon longer (60 minute) treatment times may be due to the reason that owing to a prolonged exposure time,

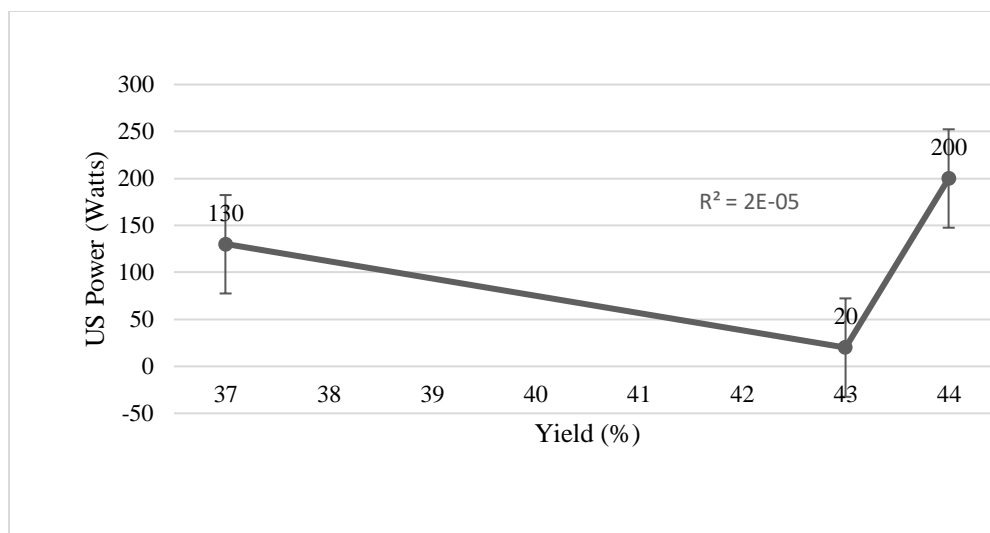
ultrasound oxidation of the edible oils take place either by thermal degradation or sonolysis which can be attributed to the phenomenon of “cavitation”. Cavitations are micro-mechanical shocks affecting the structural and functional components of the cells and such an increased phenomenon in the cells leads to lipid oxidation and deterioration (Jahouach-Rabal et al. 2008). This phenomenon may have resulted in a slight decrease in the oil yield upon increasing the ultrasound time. Further, an increase in the rate of cavitation leads to excessive breakdown of the cell membranes and cell walls which exposes the hydrophobic fatty acid tail of the phospholipids. This might result in binding of the oil to the phospholipid and thus not get extracted by the solvent. Thus, excessive ultrasound can have a delirious effect to the extractability.

#### 4.3.2. Effect of Ultrasound Power on the yield of oil

For this study, the ultrasound times were fixed at 30 minutes and 60 minutes respectively. Ultrasound powers of 20 W, 130 W and 200 W were used for US exposure time of 60 minutes. On the other hand, powers of 130 W and 200 W are used for US time of 30 minutes.



**Figure 4.9: Relation between extraction yield and US power for US time of 30 min (n=2)**



**Figure 4.10: Relation between extraction yield and US power for US time of 60 min (n=2)**

The results show that with an increase in Ultrasound power, the extraction yield of the oils increase. The maximum yields obtained are 46.8 % and 44.9 % for 30 min and 60 min respectively and are produced at an US power of 200 W. The reason for this lies in the fact that when the ultrasound power is low, the energy produced by the ultrasound probe is not enough to cause the propagation of ultrasound pressure waves to result in the cavitation phenomenon to release the oils present in the cells. The results obtained are at par with previous research led by Lin et al. 2008, who also showed that the maximum extraction yield is achieved for an US power of 200 W.

The slight decrease in the Yield % for ultrasound power of 130 W for US time of 60 minutes may be again attributed to sonolysis and the cavitation phenomenon by which the phospholipid cell membrane of the lipid bearing molecules break down exposing the hydrophobic fatty acid tail which in turn sticks to the lipid molecules thus reducing the extractability of the oil by the solvent.

#### **4.3.3. Combined Effect of Ultrasound time and power on the extraction yield of oil**

For the experiment, the solvent to solid ratio was taken as 10:1 i.e. 250 ml solvent for 25 g of the solute and the pulse-pulse ratio was maintained at 0.5. The results are illustrated in the Table 4.1.

**Table 4.1: Combined effect of US time and power on the Extraction Yield (%)**

<b>Ultrasound Power (Watts)</b>	<b>Time (min)</b>	<b>Extraction Yield (%)</b>
20	60	43
80	30	47.4
130	10	32.57
130	30	38
130	60	37.08
200	10	33.6
200	30	46.8
200	60	44.9

The maximum extraction yields were obtained for US power of 200 W for 30 min as well as US power of 80 W for 30 min which is proven by the previous results.

#### **4.4. Microwave Extraction**

The effect of two independent properties- MW temperature and MW time have been studied to understand the extraction yield. Microwaves work by rupturing and damaging cell walls as well as the lipo-protein membrane surrounding the individual lipid bodies, thus making its release easy from inside of the cell (Hu et al. 2017). For experimental purposes, we took a solvent to solid ratio of 10:1 (Rezvankah et al. 2019).

As evident from Table 5.2, with subsequent increase in the time and temperature of the MW extractor, the extraction yields also increased. The yield increased drastically from 40.31% (w/w) for MW time of 10 min to about 47.43% (w/w) for MW time of 15 min and finally to obtain the maximum yield of 53.77 % (w/w) for a MW time of 30 min. The slight decrease in the extraction yield for the microwave temperature and time combination of 70°C for 20 minutes might be attributed to the fact that owing to the elevated temperature of the solution for a longer period of time, the phospholipid cell wall and cell membrane disintegrates exposing the fatty acid tail which

in turn binds with the free oil and thus decreases the extractability of the oil. The slight decrease in the extraction yield can also be attributed to the partial thermal decomposition of the extracted oils as a result of exposure to microwaves at an elevated temperature for a prolonged period of time (Rezvankah et al. 2019, Hu et al. 2017).

This is however not observed when the MW reaction time is low (10 min). Also, contrary to the research conclusions formulated by Rezvankah et al., MW time does play a significant role in the extraction yield when the MW temperature is fixed.

**Table 4.2: Effect of MW Time and Temperature on the Extraction Yield**

<b>Time (min)</b>	<b>Temperature (°C)</b>	<b>Yield (%)</b>
10	70	40.31
15	65	47.43
20	70	45
30	65	53.77

#### **4.5. Effect of Cold pressing on the extraction yield**

The oils were obtained by cold pressing maintaining the same process parameters for each of our three trials. The extraction yield was calculated to be  $41.07 \pm 0.45$  % (w/w).

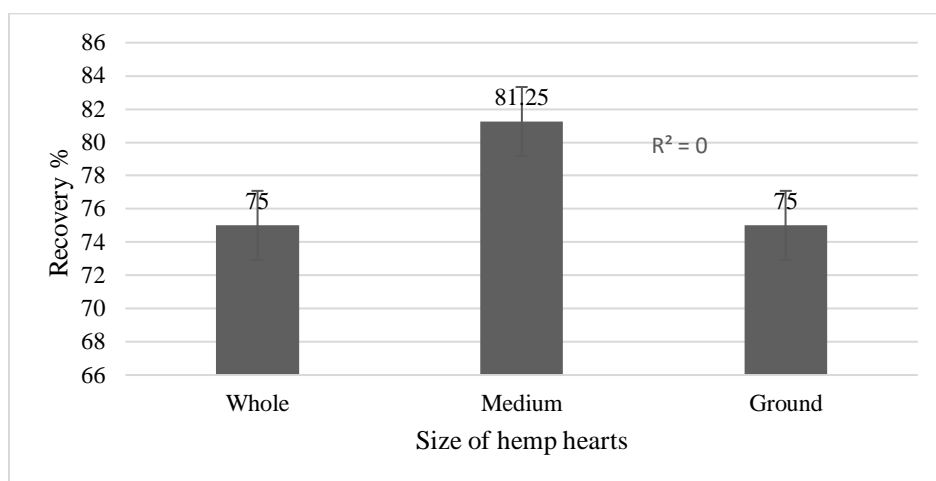
The % yield from the hemp hulls was reported to be 2.87 % (w/w) which indicated the oil contribution by the hemp hulls can be considered negligible and hence we can assume that all of the oil was extracted from the hemp hearts only. The amount of residual oil in the press cake for each of the three trials was reported to be  $6.47 \pm 0.2$  % (w/w). Further comparison between the extraction yields obtained by different extraction techniques have been illustrated and compared in Section 4.7.

#### **4.6. Comparative analysis of solvent recovery coefficient for each extraction technique**



#### 4.6.1. Conventional Soxhlet Extraction

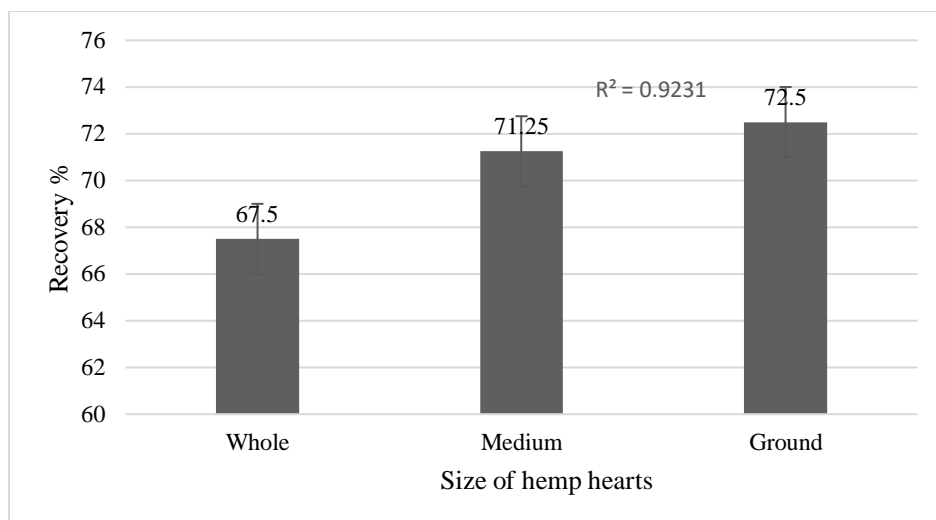
Solvent recovery is a measure of the amount of solvent that has been recovered after the extraction experiment was over. It gives an idea of the amount of solvent that can be saved and reused thus reducing the cost of the solvent. Also, since hexane is not a green solvent, if not disposed properly, it can lead to environmental pollution. Hence, recovery of the residual solvent is of utmost importance. The influence of seed size on the solvent recovery coefficient for conventional Soxhlet extraction has been shown in the form of a bar chart in Figure 4.11. It is noted that the maximum solvent recovery co-efficient is obtained for medium sized seeds at 81.25%, followed by 75% for both whole and ground seeds.



**Figure 4.11: Effect of seed size on solvent recovery co-efficient for Soxhlet extraction (n=2)**

#### 4.6.2. MW Pretreated Soxhlet extraction

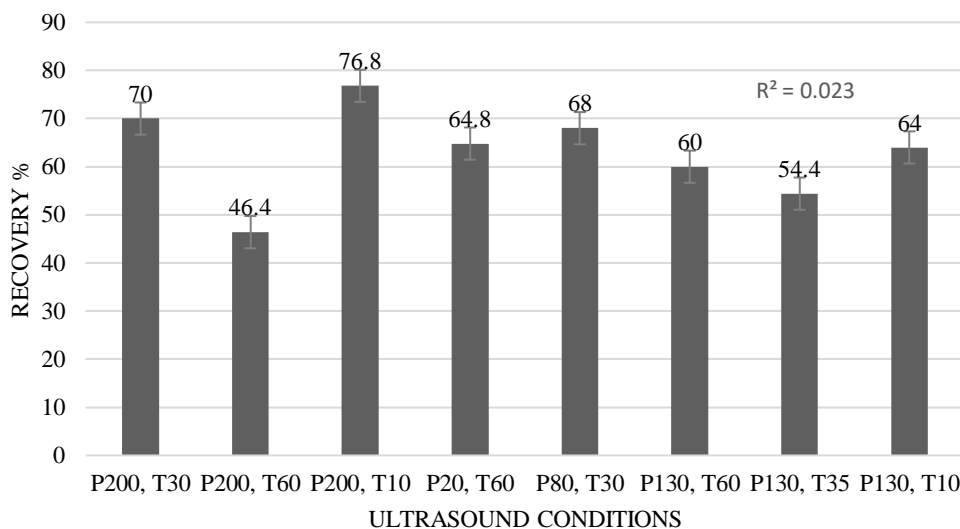
The solvent extraction co-efficient for MW pretreated Soxhlet extraction with respect to the seed size is represented in Figure 4.12.



**Figure 4.12: Effect of seed size on solvent recovery co-efficient for MW Pretreated Soxhlet extraction (n=2)**

#### 4.6.3. Effect of Ultrasound conditions on the Solvent Recovery Coefficient

The effect of different ultrasound conditions upon the solvent recovery percentage is illustrated in the bar chart in Figure 4.13.

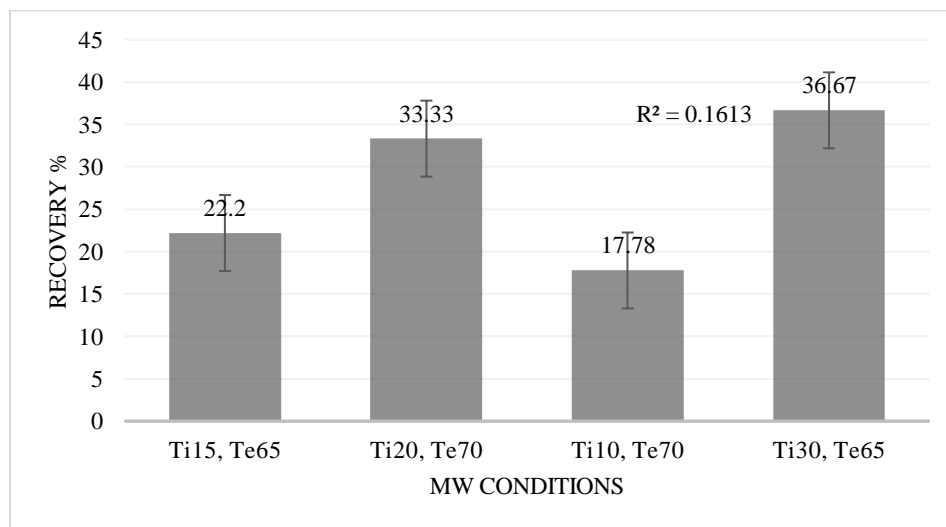


**Figure 4.13: Effect of US conditions on the solvent recovery co-efficient. T and P represent the US time (min) and US Power (Watt) respectively (n=2)**

It is noted that the maximum recovery percentages are noted for Ultrasound conditions of P200, T30; P200, T10 and P80, T30 respectively.

#### 4.6.4. Effect of Microwave conditions on the Solvent recovery co-efficient

The effects of different Microwave conditions on the solvent recovery has been studied in this section. The effect of different process parameters like MW time and temperature on the solvent recovery coefficient has been shown by a bar chart in Figure 4.14.

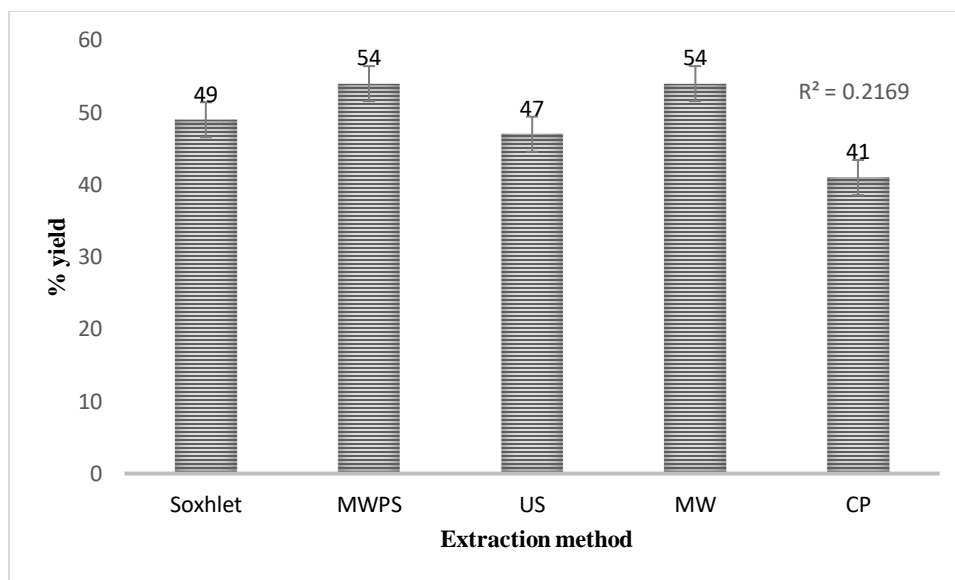


**Figure 4.14: Effect of different MW conditions on the solvent recovery co-efficient. Ti and Te stand for the MW time and MW power respectively (n=2)**

The figure illustrates that the maximum solvent recovery is achieved when the MW time is 30 minutes and the temperature of operation is 65 °C.

#### 4.7. Effect of optimum conditions for different extraction techniques on the extraction yield

A comparison was made between the different extraction techniques to compare the yield and also find the technique producing the maximum yield %.



**Figure 4.15: Extraction yield produced by different extraction techniques**

The conditions producing the maximum extraction yields was chosen from each technique and compared among each other to understand the most efficient technique in terms of yield %. It was observed that, both MWPS and MW extractions produced the maximum oil yield at  $54 \pm 4.7\%$  (w/w) followed by  $49 \pm 4.7\%$  for SE,  $47 \pm 4.7\%$  for US, and  $41 \pm 4.7\%$  for CP. This can be credited to the fact that microwaves cause increased rupture of the cell walls resulting in lipolysis and proteolysis of the cell wall materials and thus enhancing the penetration of the solvent inside the cells to extract the oils. Moreover, as discussed in Table 4.2, a temperature of 70 °C and MW time of 15 minutes (optimum condition for MW extraction) led to a movement in the media due to the reduction in solvent viscosity which enhanced the extraction yield (Kostić et al., 2013; Rezvankhah et al. 2019). Although, Rezvankhah's research showed the extraction yield in case of SE was more compared to MW extraction, however the run time reported was 8 hours which perhaps led to the increased extraction yield (Rezvankhah et al. 2019). The mechanical effect of ultrasound although promotes the release of soluble compounds from the plant material disrupting the cell walls, enhancing mass transfer and facilitating the solvent to access the cellular contents (Cravotto et al.

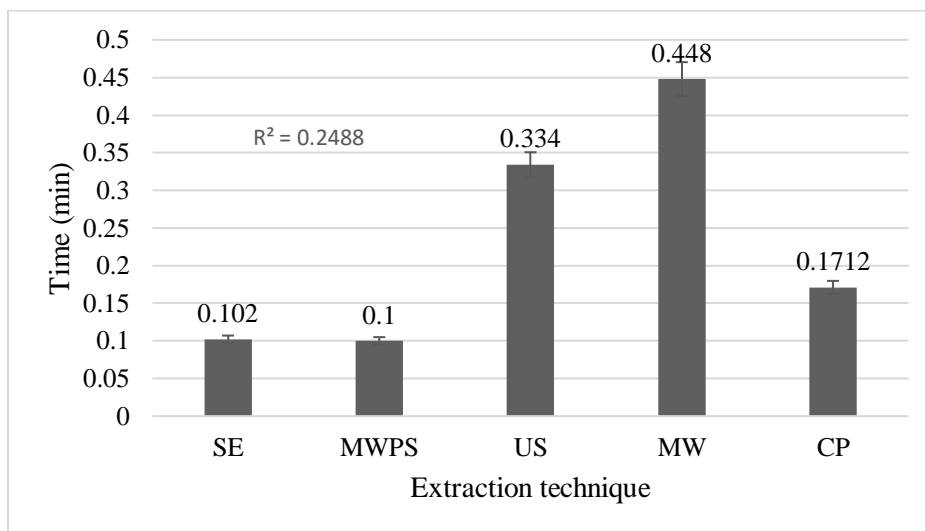
2008), microwaves lead to an increased solvent penetration into the matrix as a result of the movement of dissolved ions (since microwaves promote the rotation of molecular dipoles by disrupting the weak hydrogen bonds) and as a result causes increased extraction of intracellular materials. Also, ultrasounds may have caused the lipoprotein cell membrane to break and retain some of the oils, thus reducing the extractability. MWPS underwent a combined effect of both microwave pretreatment as well as traditional Soxhlet extraction which caused more disruption of the cell walls leading to an enhanced extraction yield.

#### 4.8. Comparison between the extraction rates among different extraction techniques

The extraction rates for each extraction method was compared to find the most time-effective extraction technique.

The extraction rate was calculated as follows:

$$\text{Extraction rate} = \text{Weight of the extracted oil (g)} / \text{Total time of reaction (min)} \dots \dots \dots \text{Equation 4.9}$$



**Figure 4.16: Comparative analysis of the extraction rate for different extraction techniques**

From our experiments, it is noted that, the extraction rate for SE and MWPS are the least and was  $0.1 \pm 0.002$  g/min. On the other hand, the amount of oil extracted per unit time for MW extraction was the maximum at 0.448 g/min followed by US at 0.334 g/min. This can be attributed to the

rupture of cell walls due to microwaves and ultrasound which caused an increased exposure of the intracellular materials to the solvent, resulting in enhanced extraction over a shorter period of time. The only limitation that lies in this case are that the extraction times deployed for each of the reaction was not the same. The extraction rates have been calculated based on the experiments which gave the maximum oil yield for each extraction technique. Thus, calculating the extraction yield on the basis of a specific time for each technique can be considered as a future scope of study.

#### **4.9. Gas Chromatography Mass Spectrometry analysis of Oils obtained**

GC-MS analysis of the oil samples obtained from different extraction techniques was performed to analyze the differences in the composition of Fatty acids, Tocopherols and other minor compounds present in the oil. The major compounds present in the oils are presented in Table 4.3.

The present study compared the GC-MS results based on FA profiles for each process. The main components found in our samples consisted of linoleic acid, trilinolein, palmitic acid and oleyl oleate. (Aladić et al. 2015; Da Porto et al. 2012; Devi and Khanam 2019). From the GC-MS values, the main component present in the oils is found to be the triglyceride Trilinolein with a relative area % of 60.29, 55.43, 49.65 and 43.16 for oils obtained by SE, MWPS, US and CP, respectively (90% identity when compared with the National Institute of Standards and Technology library). Trilinolein is a naturally occurring triglyceride which has anti-isthmia, anti-arrhythmic and antioxidant properties (Chan et al. 2005) and has found profound uses in the pharmaceutical and food industry. It has also been used in cosmetics.

Linoleic acid (LA/n-6 PUFA) is the second most significant compound present in the extracted oils with a % of 13.99, 14.49, 8.94 and 12.08 for TS, MWPS, US and CP, respectively. Here we notice that the relative percentage of trilinolein is more in case of TS as compared to MWPS whereas, the relative area percentage of LA is more in the case of MWPS. This can be

attributed to the effect of microwaves which might have caused hydrolysis of the trilinolein molecule into linoleic acid. US extracted oil showed a lower % of LA which is similar to the results obtained by Devi and Khanam (2019).

**Table 4.3: GC-MS analysis of hemp oil samples**

Name of compound	Retention time	Relative area %				Molecular Formula
		TS	MWPS	US	CP	
Trilinolein	49.646	60.29	55.43	49.650	43.16	C <sub>57</sub> H <sub>98</sub> O <sub>6</sub>
Linoleic acid	30.118	13.99	14.49	8.94	12.08	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>
Palmitic acid	27.677	nd	nd	1.55	nd	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>
Oleanolic acid	45.488	nd	nd	nd	0.59	C <sub>30</sub> H <sub>48</sub> O <sub>3</sub>
Oleyl oleate	45.395	1.66	1.29	1.85	nd	C <sub>36</sub> H <sub>68</sub> O
Betulin/Lupeol	42.616	tr	0.85	0.955	0.82	
Rhodoxanthin	43.121	0.24	nd	nd	2.99	C <sub>40</sub> H <sub>50</sub> O <sub>2</sub>
Psi, psi Carotene	37.716	nd	0.3	nd	nd	C <sub>40</sub> H <sub>66</sub>
Methyl cholate	59.39	nd	0.46	nd	nd	C <sub>25</sub> H <sub>42</sub> O <sub>5</sub>
Demecolcine	53.294	nd	nd	1.09	1.04	C <sub>21</sub> H <sub>25</sub> NO <sub>5</sub>
Stigmasterol	41.512	0.38	nd	nd	nd	C <sub>29</sub> H <sub>48</sub> O
Decanedioic acid	51.557	nd	0.83	nd	nd	C <sub>36</sub> H <sub>66</sub> O <sub>8</sub>
γ-Tocopherol	39.768	2.75	2.62	3.64	1.83	C <sub>28</sub> H <sub>48</sub> O <sub>2</sub>
γ-Sitosterol	41.995	8.64	7.64	7.955	7.04	C <sub>29</sub> H <sub>50</sub> O
Octasiloxane	54.803	nd	nd	nd	2.16	
Methyl glycocholate	54.468	nd	nd	nd	3.54	C <sub>36</sub> H <sub>69</sub> NO <sub>6</sub> Si <sub>13</sub>

This may be a result of the large duty cycle and amplitude resulting in a degradation of the quality. This PUFA is essentially important in the cardiovascular health. According to the data from clinical studies and meta-analyses by Marangoni et al. (2020), there is a positive co-relation between high dietary intakes of LA and the improvement of cardio-vascular health. It also

influences the long-term glycemic control and insulin resistance (Marangoni et al. 2020). Latif et al. (2009) and Ribnicky et al. (2014) also reported the presence of Linoleic acid in hemp seed oils. Presence of Palmitic acid (n-16) in very low amounts and oleanolic acid has been noted for US and CP oils respectively. Palmitic acid is a saturated Fatty acid and although its effect on human health has been considered detrimental a recent study showed that a balanced intake of Palmitic acid with other n-6 and n-3 PUFA is essential to maintain the membrane phospholipid balance (Carta et al. 2017). Oleyl oleate which has been reported in TS, MWPS and US oil samples has found profound uses in the cosmetic industry in lotions, creams, lipsticks and is essential for lubrication. Minute quantities (0.83 %) of decanedioic acid have also been reported in MWPS oil. Apart from the presence of fatty acids and triglycerides, numerous other compounds like sterols, vitamins and carotenoids have also been reported in the oils. Of them, the notable ones are  $\gamma$ -Sitosterol,  $\gamma$ -Tocopherol, stigmasterol, rhodoxanthin, carotene, methyl cholate, methyl glycocholate, demecolcine. Their ranges of application are varied.  $\gamma$ -Sitosterol has anti-bacterial and anti-fungal properties (Hernandez-Hernandez et al. 2017), Stigmasterol, a phytosterol has been documented as an immunomodulator which possesses a great therapeutic potential (Antwi et al. 2017). Overall, the results give us a generalized GC-MS scan of the components present in our experimental oil samples. The results indicate that the components present in the oils vary in composition and depending upon the different extraction techniques adapted may result differently due to nature of heating mechanisms involved as well as other physical treatments followed. The presence of minor components also indicates to some extent that the level of fatty acid was low.

#### **4.10. NMR Spectroscopic Characterization of Hemp heart Oils**

The signals in the NMR spectra have been previously assigned by Vigli et al. 2003 and Popescu et al. 2015. The chemical groups generating the signals and their chemical shifts in  $\text{CDCl}_3$  solvent



have been tabulated in Table 4.4.

**Table 4.4: Assignment of the main resonances in  $^1\text{H}$  NMR Spectrum of vegetable oils**

Chemical shift $\delta$ (ppm) (No.)	$^1\text{H}$	Compound
0.87 (1)	$-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$	All acids except linolenyl
1.02 (2)	$-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}_3$	Linolenyl
1.30 (3)	$(\text{CH}_2)_n$	All acyl chains
1.62 (4)	$-\text{CH}_2-\text{CH}_2-\text{COOH}$	All acyl chains
2.03 (5)	$-\text{CH}_2-\text{CH}=\text{CH}$	All unsaturated fatty acids
2.32 (6)	$-\text{CH}_2-\text{COOH}$	All acyl chains
2.77 (7)	$-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}=\text{CH}-$	Linoleyl and linolenyl
4.22 (8)	$-\text{CH}_2-\text{OCO}-\text{R}$	Glycerol (Triacylglycerol)
5.26 (9)	$\text{CH}-\text{OCO}-\text{R}$	Glycerol (Triacylglycerol)
5.37 (10)	$-\text{CH}=\text{CH}-$	All unsaturated Fatty acid

**Table 4.5: Empirical formulas for calculation of the composition of Fatty acids**

No.	Parameter/ Fatty acid	Relationship
1	Linolenic	$\frac{(2)}{(2) + (1)}$
2	Linoleic	$\frac{(3) \cdot (7) - (4)(2)}{2 \cdot [(2) + (1)]}$
3	Oleic	$\frac{(3) \cdot (5)}{(4)[(2) + (1)]} - [\text{Linolenic}] - [\text{Linoleic}]$
4	SFA	$\frac{(1)}{(2) + (1)} - [\text{Linolenic}] - [\text{Linoleic}] - [\text{Oleic}]$

The integrals of the  $^1\text{H}$  NMR values is strictly proportional to the number of hydrogen atoms present in each of the functional groups as well as the number of functional groups present in the sample. From the  $^1\text{H}$  NMR spectra of our oil samples, the main FA concentrations can be calculated by combining various signal integrals from Table 5.6 using the empirical formulas stated in Table 4.5 (Vigli et al. 2003; Mannina et al. 2003).

**Table 4.6: Integrals of the  $^1\text{H}$  NMR main resonance groups for the different hemp heart oil samples**

Peak no.	1	2	3	4	5	6	7	8	9	10
<b>Ppm</b>	0.87	1.02	1.3	1.62	2.03	2.32	2.77	4.22	5.26	5.37
<b>TS</b>	10.66	2.7	135.26	18	15.8	16.65	8.55	5.41	1.05	16.1
<b>MWPS</b>	10.4	3.1	155	26.2	16.2	17.8	8.8	5.7	1.04	15.7
<b>MW</b>	10.3	2.35	199	24.21	15.27	17.3	8.5	5.6	1.03	15
<b>US</b>	9.9	3.1	220.2	26.7	15.8	18.3	9.07	5.8	1.05	15.8

**Table 4.7: Oil samples composition (% molar)**

Name of extraction technique	Linoleic acid (% molar)	Linolenic acid (% molar)	Oleic acid (% molar)	SFA (% molar)	Ratio of LA: ALA	Ratio of UFA: SFA
Soxhlet	55.58	20.21	12.91	11.3	2.75	6.7
MW Pretreated Soxhlet	51.85	22.96	15.19	10	2.25	7.5
Microwave	63.64	18.58	8.32	9.47	3.42	8.7
Ultrasound	56.96	23.85	10.35	8.85	2.4	9.1

From Table 4.7, it is evident that Linoleic acid (LA) was the major PUFA calculated by  $^1\text{H}$  NMR. The maximum concentration of LA was calculated for MW (63.64 mol %) followed by US (56.96 mol %), TS (55.58 mol %) and MWPS (55.85 mol %). Apart from LA, alpha Linolenic acid or ALA was also found to be present in the hemp oil samples in minor quantities ranging from 18.58 mol % for MW extracted oils to 23.85 mol % for US oils. The health benefits of ALA ( $\omega$ -3 FA) cannot be ignored. Studies have repeatedly shown that they have significantly helped in enhancing the heart health (Su et al. 2003; Balanza-Martinez et al. 2011). Moreover, they have found to improve numerous mental disorders like schizophrenia, depression and bipolar disorder (Ghosh et

al. 2007, Lagarde et al. 2018). It also has inhibitory effects on cancer and tumor growth (Leizer et al. 2000). The consumption of  $\omega$ -6 and  $\omega$ -3 Fatty acids in a ratio of 3:1 is considered optimal for nutrition (Leizer et al. 2000; Paz et al. 2014). The ratios of LA and ALA in our samples was close to 3:1 and hence they can be considered optimal for nutrition. Moreover, literature survey states that, a LA:ALA ratio of 2.5:1 helped in the reduction of rectal cell proliferation in patients with colorectal cancer, whereas a ratio of 4:1 with the amount of  $\omega$ -3 being unchanged had no effect. Also, a lower  $\omega$ -6: $\omega$ -3 ratio in women with breast cancer was associated with a decreased risk and a ratio of 2-3:1 suppressed inflammation in patients. A higher ratio of 10/1 on the other hand was found to produce adverse effects (Artemis 2008). The concentration of SFA was also found to be very less which indicates that hemp seed oil is suitable for human consumption. The ratio of unsaturated fatty acids (UFA) to saturated fatty acids (SFA) also varies between 6.7-9.1. This high ratio is considered favorable for reducing blood cholesterol and preventing cardiovascular diseases. A MUFA, oleic acid was also found in the hemp seed oil samples ranging between 8 and 13 mol %.

#### **4.11. Effect of different Extraction techniques on the antioxidant profile of hemp seed oil**

##### **4.11.1. Tocopherols**

Vitamin E has been studied to function primarily as a chain-breaking antioxidant and it prevents the propagation of lipid peroxidation. GC-MS studies of our oil samples have shown the presence of  $\gamma$ -Tocopherol.  $\gamma$ -Tocopherol or Vitamin E possess anti-inflammatory properties and might be beneficial in the treatment of cardio-vascular disease and prostate cancer (Jiang et al. 2001) It also has antioxidant effects in very low quantities (Lampi et al. 1997). Although previous studies by Latif et al. and Anwar et al. have shown the presence of  $\alpha$ ,  $\gamma$  and  $\delta$  Tocopherols in hemp oil samples from Pakistan, the quantities of  $\alpha$  and  $\delta$  tocopherols have been significantly less compared to  $\gamma$ -

tocopherols. Again, according to Oomah et al. 2002, the major Tocopherol present in hemp seed oils is  $\gamma$ -Tocopherol. Vitamin E profile of oil extracted from hemp seeds can also be compared to those of peanut oil, olive oil as well as soybean oils. (Ensminger et al. 1993).

#### 4.11.2. $\beta$ -Carotene

From spectrophotometric analysis, minute quantities of  $\beta$ -Carotene have been reported in our hemp oil samples which are shown in Table 4.8.

**Table 4.8: Quantity of  $\beta$ -Carotene present in hemp seed oil**

Method of extraction	$\beta$ -Carotene (mg/100g)
US	0.375
MW	1.662
MWPS	1.287
TS	0.375

Oomah et al. 2002 also reported very small quantities of  $\beta$ -Carotene in hemp seed oils cultivated in Manitoba which lie in accordance with our results. Carotenoid content of hempseed oils in his experiments also increased significantly when the seeds were pre-treated with microwave. Dietary carotenoids (especially  $\beta$ -Carotene) are thought to provide health benefits in combatting certain types of cancers and eye diseases. It is also believed to have added benefits since it is converted to Vitamin A (Johnson et al. 2002).

#### 4.12. Viscosity of hemp heart oils obtained by using different extraction techniques

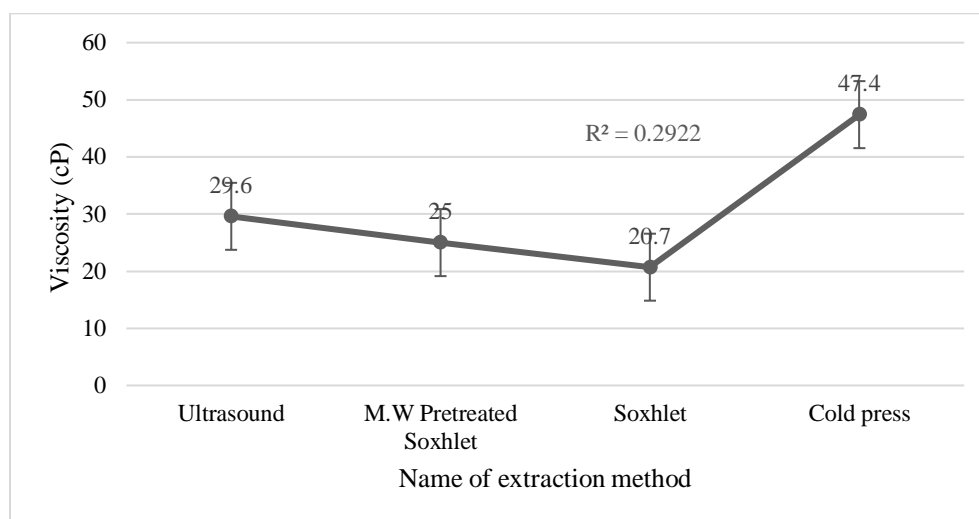
The viscosity of a fluid is a measure of its resistance to deformation at a given rate (Simon, 1971). In our experiments, the viscosity of hemp heart oils, obtained from 5 different extraction techniques have been illustrated at two different temperatures 25°C and 40 °C for two different

rpms of 3 and 6. The results obtained are illustrated in the form of graphs in Figures 4.17, 4.18, 4.19 and 4.20.

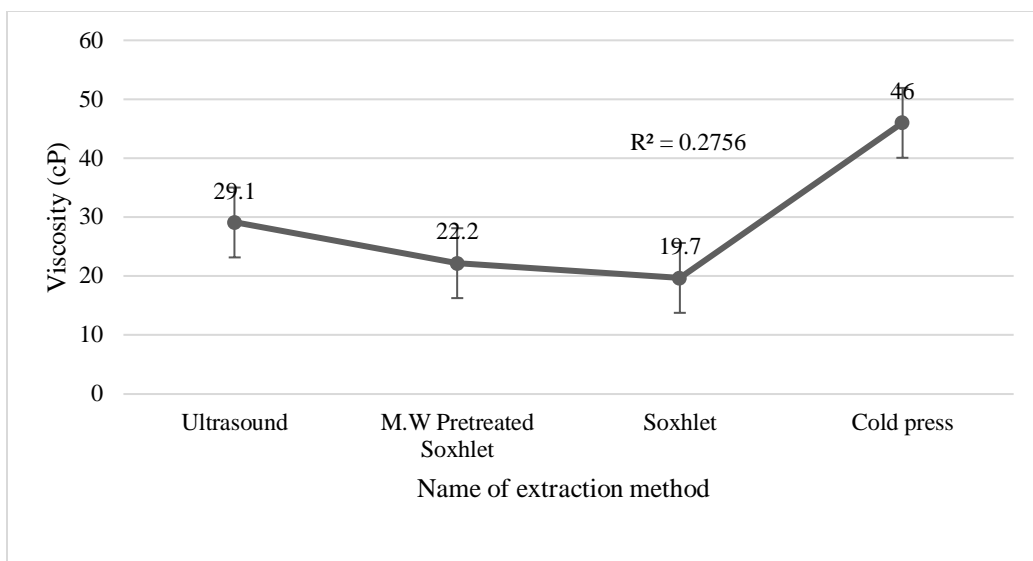
**Table 4.9: Variation of the viscosity with R.P.M**

R.P.M	% Deflection	Viscosity (cP)
0.6	4.9	25.5
1.5	13.7	28.2
3	27.9	28.7
6	59.4	30.3
12	Error	Error

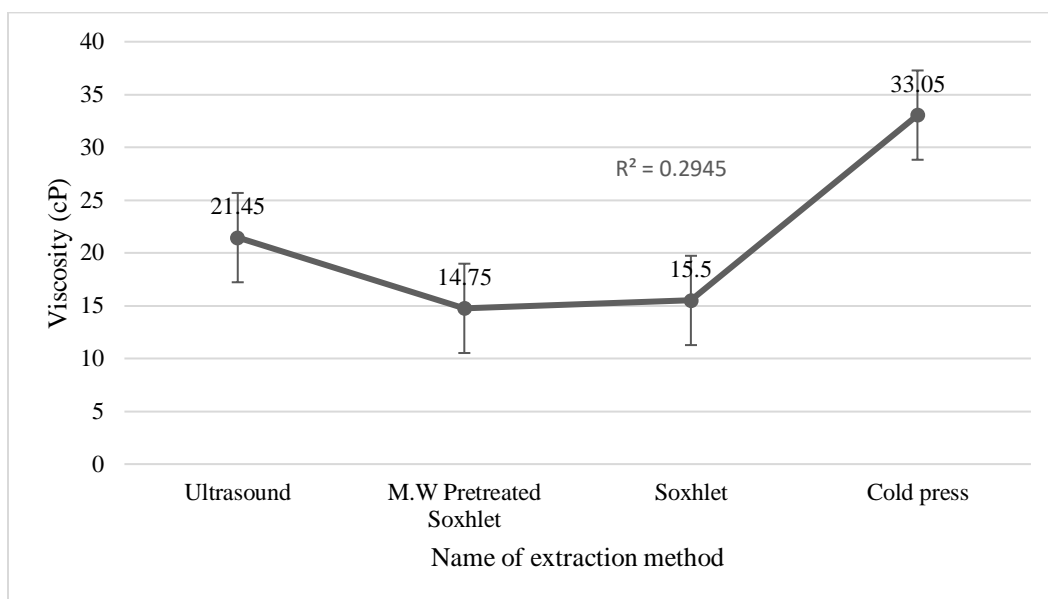
From the Newton's law of viscosity test, it can be concluded that our oil samples are Newtonian in nature since the viscosity does not vary considerably with a change in R.P.M for a particular temperature. Also, for performing our experiments, R.P.M s of 3 and 6 were chosen since they produced the best deflections.



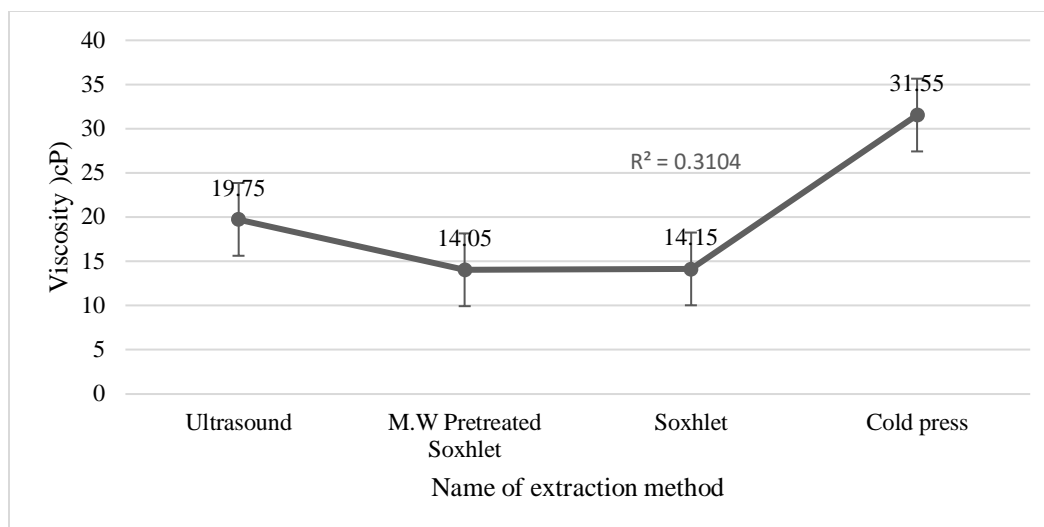
**Figure 4.17**



**Figure 4.18**



**Figure 4.19**



**Figure 4.20**

**Figures 4.17, 4.18: Graphical representation of the effect of different extraction techniques on the oil viscosity at 25 °C for R.P.M 3 and 6 respectively (n=2)**

**Figures 4.19, 4.20: Graphical representation of the effect of different extraction techniques on the oil viscosity at 40 °C for R.P.M 3 and 6 respectively (n=2)**

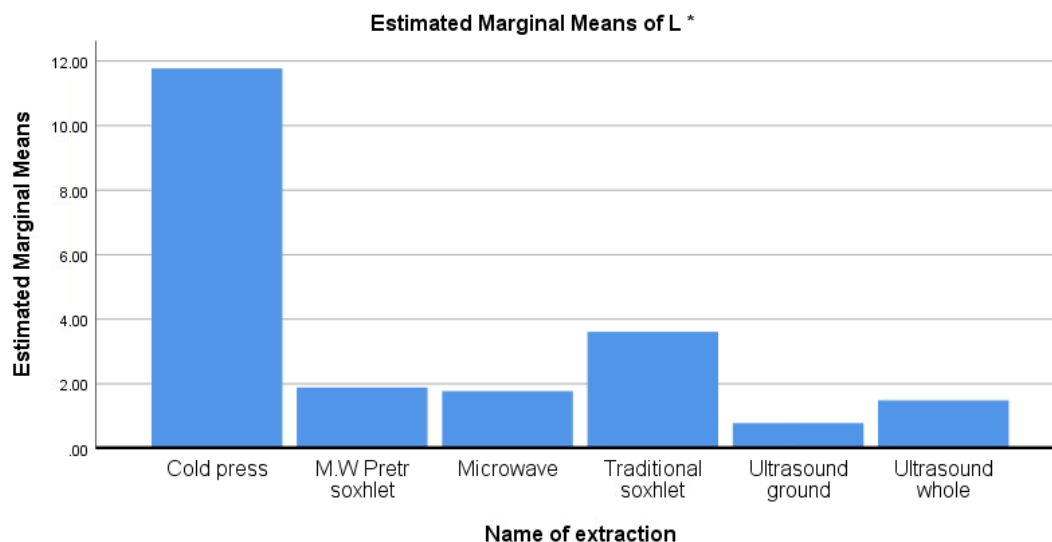
We notice that the viscosity for each of the oil decreases with an increase in the temperature. This can be analyzed from the thermodynamic perspective. As the temperature of the system increases, the molecules gain energy and the kinetic energy of the molecules increase leading to less intermolecular interactions. Thus, the viscosity decreases with an increase in the temperature.

Again, the viscosity obtained for MW Pretreated Soxhlet and conventional Soxhlet extracted oils at 25 °C are 25 cP, 22 cP and 20.7 cP and 19.7 cP respectively which are the least among the oils obtained from all the different extraction techniques. The CP oils on the other hand have the highest viscosity. Cold pressing is essentially a physical mechanism to separate the oil from the cells with the help of shear and inter-molecular elasticity, and pressure. Thus, even after centrifugation, some dispersed or slightly soluble phospholipids, lipoproteins, minerals and polysaccharides might still be present in the extracted oil (cruder) which makes it more viscous when compared to the other oils.

The same trend is also observed at temperatures of 40 °C, where the viscosity recorded for oils extracted by SE and MWPS are more than oils extracted by CP. Moreover, viscosity is one of the main factors governing oil absorption and drainage (Ziaiifar et al. 2008) i.e. more is the viscosity of oil, slower is the oil drainage. The viscosity reported in our samples vary between 20 cP and 50 cP at a temperature of 25 °C and 15 cP and 35 cP at 40 °C which can be considered a bit low since vegetable oils like canola, olive, corn, peanut and soybean usually have viscosity in the range of 55-75 mPa at 20 °C and in the range of 30-45 mPa at 40 °C (Ziaiifar et al. 2008).

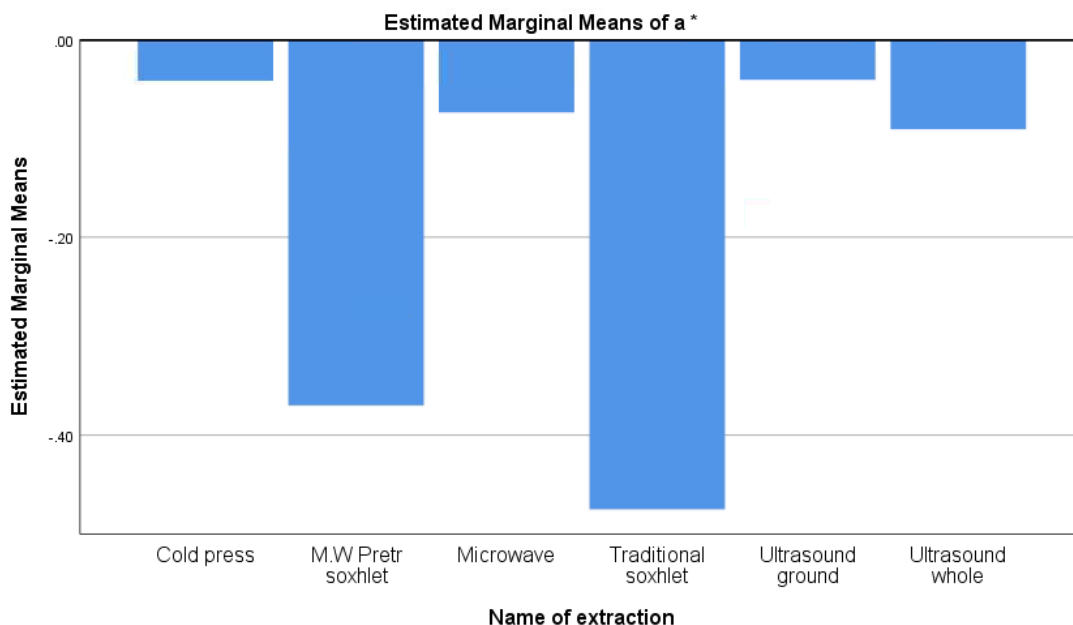
#### 4.13. Effect of different extraction techniques on the color of oils

The color measured was expressed in terms of L, a and b values where L gives a measure of the lightness of the sample, “a” the ratio of red/green color in the sample and “b” the ratio of yellow/blue co-ordinate. The comparison between the L, a and b co-ordinates of the oil samples are shown in Figures 4.21, 4.22 and 4.23.

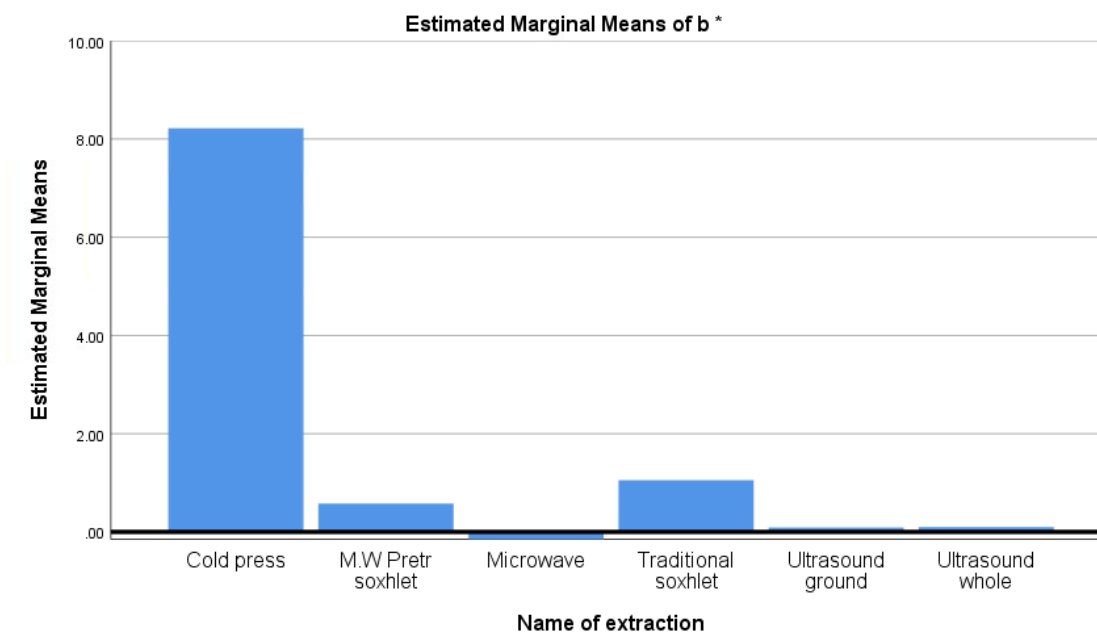


**Figure 4.21: Comparison between the mean lightness (L) values of the oil samples obtained by different extraction methods (n=2)**





**Figure 4.22: Comparison between the mean red/green (a) co-ordinate of the oil samples obtained by different extraction methods (n=2)**



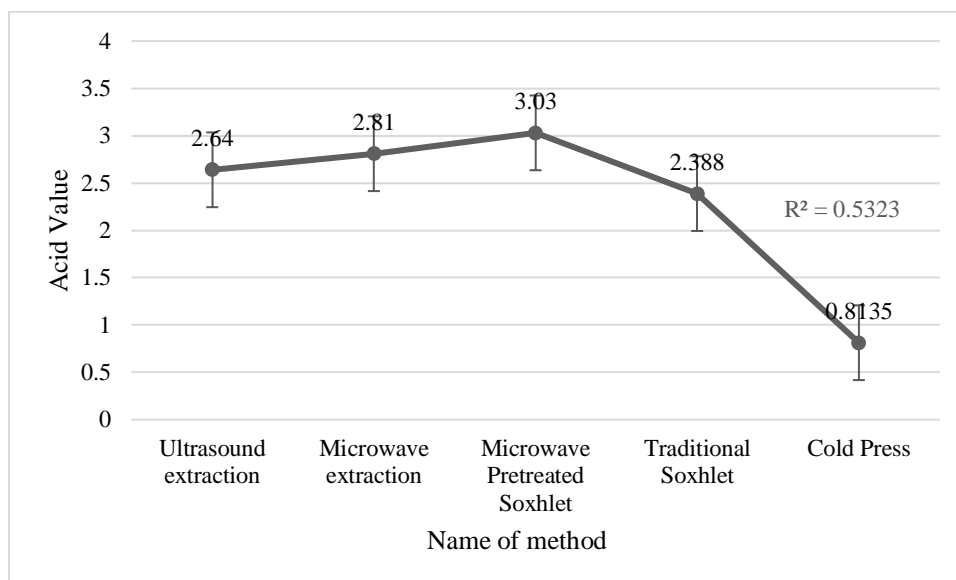
**Figure 4.23: Comparison between the mean yellow/blue (b) co-ordinate of the oil samples obtained by different extraction methods (n=2)**

From our measurements, it is clearly seen that the mean L and b co-ordinates for the cold pressed hemp oil are significantly higher when compared to the other oils. On the other hand, the mean a

co-ordinate is significantly lower on the negative Y axis. This can be attributed to the presence of chlorophyll in the oils obtained from the hemp hulls during the process of screw pressing. As a result, the mean “a” co-ordinate, which is the ratio of red/green decreases whereas the mean “b” co-ordinate which is the ratio of yellow/blue increases. The decrease in lightness or the increase in the “a” co-ordinate for oils obtained by different extraction techniques other than cold pressing may be attributed to the presence of browning substances in the oil obtained from Maillard type non-enzymatic reactions between the phospholipids and the free amino acids present in the hemp hearts causing further phospholipid degradation and darkening the color (Yen 1990; Zheng et al. 2018) during the pre-treatment or the extraction process.

#### 4.14. Acid Value of hemp heart oils

The acid value was determined by volumetric titration method following AOCS Official Standard method named Iodine Value of Fats and Oils (Wijs Solution) Cd-1-25. The acid values obtained is represented graphically in Figure 4.24.



**Figure 4.24: Effect of different extraction techniques on the AV of hemp oil (n=2)**

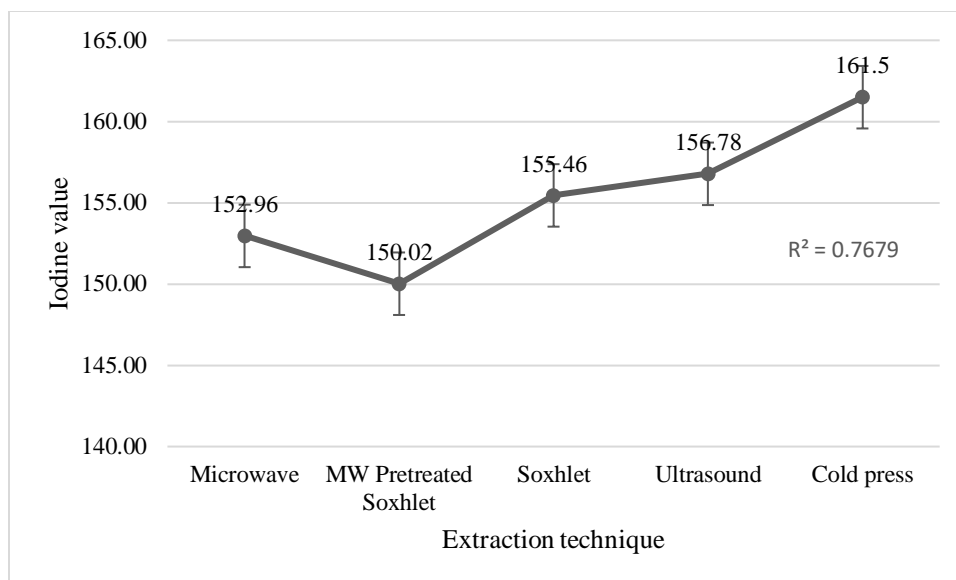
Acid value is a measure of the amount of FFA present in the oils. It gives us an idea about the

suitability of oil for its direct consumption or for industrial use (Al-Bachir, 2015). From our experiments, it is observed that the maximum A.V of 3.0335 (mg KOH/g of oil) is obtained for MW Pretreated oils followed by AV of 2.388 (mg KOH/g of oil), 2.8167 (mg KOH/g of oil) and 3.26 (mg KOH/g of oil) for SE, MW and UE respectively. The AV dropped abruptly to 0.8135 (mg KOH/g of oil) for the cold pressed oil. It can be assumed that due to the heat generated as a result of the elevated reaction temperatures for each of the extraction experiments, the hydrolytic and lipolytic activities become relatively high (Dawodu et al. 2015). This causes the triglycerides present in the oils to break down into FFA, deteriorating the oil (Dawodu et al. 2015). On the other hand, cold pressing takes place at room temperatures and hence the lipase activity was minimum compared to the other extraction techniques. MWPS extracted oils showed the maximum AV which can be attributed to the higher temperatures (combination of microwave pre-treatment and Soxhlet extraction temperatures) to which the seeds got exposed to during the extraction experiments. However, the values obtained from the experiments lie within the range of 0.6 and 10 mg KOH/g for virgin and non-virgin edible oils (Codex Alimentarius 1999; Codex Alimentarius 2001) which states that they are safe for consumption.

#### **4.15. Iodine Values of hemp heart oils**

The iodine value (IV) of SE, MW, MWPS and US oils were determined by  $^1\text{H}$  NMR spectroscopy. Figure 47 gives us an idea about the effect of different extraction techniques on the IV of the extracted oil samples.

Iodine value is an important indicator of the degree of saturation and unsaturation in the oil with saturated fats and oils having low IV and unsaturated fats and oils having high IV (Dawodu et al. 2015). Iodine value thus depends upon the number of double bonds present in the oil (Sanli et al. 2014).



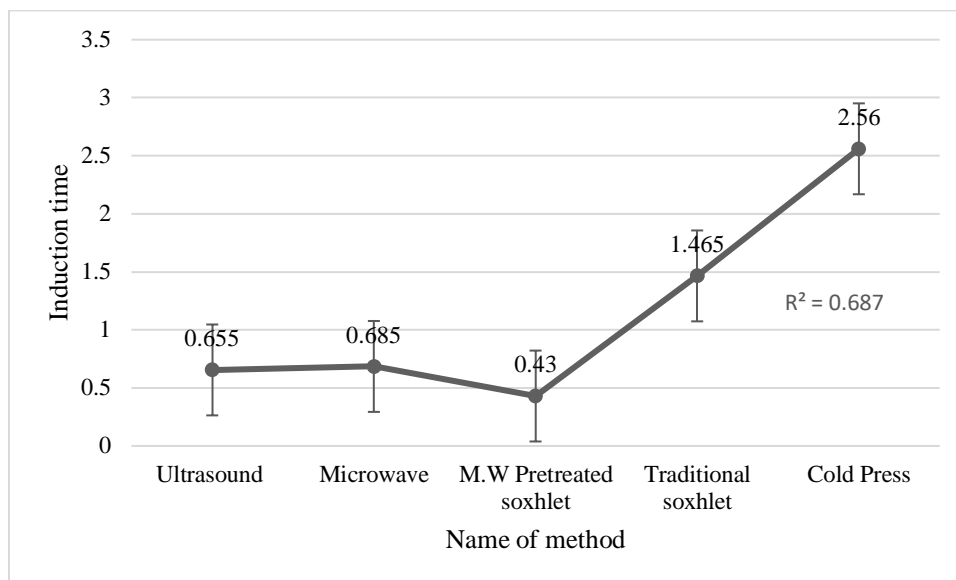
**Figure 4.25: Effect of different extraction techniques on the IV of the oil (n=2)**

In our experiments, the IV ranged between 150 and 170 which is similar to the IV of hemp seed oils obtained by Anwar et al. (2006), Latif et al. (2009) and Chen et al. (2010). The maximum IV has been recorded for CP oils whereas the minimum IV has been recorded for MWPS oils. The high IV indicates that the degree of unsaturation present in the oils is very high. On the other hand, a higher temperature and a longer extraction time especially in the case of MWPS and TS extracted oil caused some oxidative damage to the oil resulting in a lower IV. Hence, although MWPS resulted in a higher extraction yield, the composition of the oil got changed. The same conclusion can be drawn in the case of MW extracted oils where the microwave radiation caused more oxidation in the oils resulting in a lower IV.

#### 4.16. Oxidative stability of hemp heart oils

In order to determine the oxidative stability of the hemp heart oils, the Rancimat method was used as discussed in the materials and methods section in Chapter 2. During the Rancimat test, the sample was heated to 110°C and oxygen was supplied. In presence of O<sub>2</sub> at high temperatures, oxidation reaction takes place and the derivatives are transferred to the measuring chamber

containing the Millipore water. The increase in conductivity of the water was detected as the derivatives from oxidation were transferred into it. The induction time is defined as the time required for the conductivity of the water to be increased rapidly and was used as an indication of the oxidative stability of our sample.



**Figure 4.26: Effect of different extraction techniques on the Induction time of oils (n=2)**

We know that, oxidation is an exponential chain reaction process leading to the generation of free radicals. The Rancimat results in our experiments indicate that cold press oils have the maximum induction time of 2.56 hours followed by SE of 1.5 hours. MWPS on the other hand has the least induction time of 0.43 hours which can be attributed to the combined effect of elevated reaction temperature and microwave irradiation time resulting in increased oxidation.

The presence of antioxidants like tocopherols in higher concentrations in CP oils can be a reason for a higher induction time since we know that these antioxidants act as free radical scavengers and singlet oxygen quenchers. It is also known that factors like the processing and storage treatment as well as the fatty acid composition of the oil play a key role in determination of the oxidative stability. Since CP took place at room temperature (22 °C) it is highly likely that

oxidation reaction did not initiate during its storage leading to a higher induction time during the analysis.

#### **4.17. Scalability factors:**

Scalability involves design modification and improvements, switching to energy saving modes, elevated throughput capacity, safety and quality manufacturing in order to transfer the laboratory scale work onto an industrial scale, thereby increasing the production.

From our experiments, it is evident that M.W extraction is the most time-efficient method and also has the maximum extraction yield. In order to scale up, a thorough economic evaluation as well as a study of the power consumption need to be performed before transferring the laboratory scale research onto an industrial scale.

Cold pressing on the other hand is another extraction technique whose extraction yield is not that high, but the quality of oil produced by extraction is better compared to the other extraction techniques that have been discussed in the thesis. It can be scaled up more easily by using a larger screw press with a wider dye diameter. It might be more time consuming as compared to MW extraction but will be very cost-effective.

## **CHAPTER 5**

### **5.1. Summary and conclusions**

Hemp seed oil is highly valued around the world not only for its nutritional properties but also its benefits in health and skincare. As a result, its importance is booming in the food, pharmaceutical, medicine and cosmetic industries in the present day. Hemp oil has been said to possess properties comparable to or even better than existing vegetable oils like canola, maize, corn, olive, groundnut and others. Although various research reports are available on the extraction of hemp seed oils, most of them are mainly focused on either cold pressing or traditional solvent extraction or the SFE (Supercritical Fluid Extraction). This research has made a comparative analysis of the extraction efficiencies of new and emerging extraction techniques operating over wide range of temperatures. It has also shown that different extraction techniques influence the composition of the components (fatty acids, tocopherols, carotenoids, sterols and other minor phytochemicals) present in the oils. Certain minor compounds have been reported such as siloxane, methyl glycocholate, demecolcine, rhodoxanthin, carotene, betulin and lupeol in the oils which have not been previously reported and have been studied to possess health benefits. As a whole, it can be concluded that the hemp oils obtained in our research are of improved (good) quality and hence can be of benefit to the food, pharmaceutical and higher value nutraceutical industries.

The salient conclusions achieved in this research are as follows:

#### **First Objective:**

1. Different extraction techniques like the conventional Soxhlet extraction (SE), Microwave-pretreated Soxhlet extraction (MWPS), Ultrasound extraction (US), Microwave extraction (MW) and Cold pressing (CP) have been used for extracting oils from de-hulled hemp seeds. The effect of different extraction parameters on the extraction efficiency for each extraction technique has

been studied in detail. Studies showed that for SE, MWPS and US, the smallest particle size of 0.85 mm and less (ground seeds) yielded the maximum oil whereas the largest particle size of 1.4 mm and more (whole seeds) yielded the minimum quantity of oil. This can be attributed to the fact that, smaller the size of the particle, the more is the contact surface area between the solvent and the seeds and hence better extraction takes place.

2. For US extraction, a power of 200W for 30 min exposure time and a power of 80W for an exposure of 30 min time produced the maximum extraction of around 47 % in each case.
3. For MW extraction, the maximum yield of 53.77 % was achieved by operating the MW at a temperature of 65 °C for 30 minutes.
4. The maximum solvent recovery (81.25 %) was recorded for SE of medium sized seeds.

### **Second objective**

The oils were analyzed using the GC-MS and NMR to find the composition of Fatty Acids, antioxidants and other nutritionally and medically important compounds. Thereafter, further analysis was performed to find the physico-chemical properties of the oil like the color, viscosity, acid value, iodine value and oxidative stability.

1. Trilinolein, a naturally occurring triglyceride was found to be present in the oil which has pharmacological effects in the form of antioxidants.
2. The major poly unsaturated fatty acid (PUFA) extracted from the oil was  $\omega$ -6 fatty acid Linoleic Acid which has been reported to reduce inflammation and also whose consumption in moderate levels is good for cardiac health.
3. Another important FA found in smaller amounts was the  $\omega$ -3 PUFA  $\alpha$ -Linolenic acid which has been said to have significantly positive effects on the heart health, tumor growth, breast cancer as well as mental disorders.



4. The ratio of Linoleic acid (LA): $\alpha$ -Linolenic acid (ALA) found in our samples is very close to the ratio of 3:1 which is recommended as optimal for human intake.
5. The ratio of LA:ALA found in our CP, MWPS and US oils is considered ideal for the reduction of inflammation.
6. The ratio of unsaturated fatty acids (UFA) and saturated fatty acids (SFA) is very high and lies in the range of 6.7-9.1 making it very appropriate for human consumption having an excellent balance of both SFA and UFA.
7. Palmitic acid, a saturated fatty acid has also been reported in our oil samples which taken in moderation with unsaturated fats can increase the nutritional value of our food as well as maintain the membrane phospholipid balance in our cells.
8. Oleyl oleate reported in our oils has profound uses in the cosmetic industry.
9. Antioxidants like  $\beta$ -Carotene and  $\gamma$ -Tocopherol (Vitamin E) present in our samples protect our body from free radicals and play an important role in the cardiac health as well as treatment of cancer, eye disorders and other diseases.
10. Various other smaller compounds have also been detected in our oil samples like  $\gamma$ -Sitosterol, stigmasterol, rhodoxanthin, carotene, methyl cholate, methyl glycocholate, demecolcine which have not been previously reported.
11. Just like other vegetable oils, the viscosity of hemp seed oil also decreases with an increase in temperature and it can also be fitted in an Arrhenius type equation proving that it is Newtonian in nature. Moreover, except viscosity of CP hemp seed oils, viscosities of all the other oils were in the range of 20 cP and 50 cP at 25 °C and 15 cP and 35 cP at 40 °C which is a bit low compared to some important cooking oils like canola, olive and corn oils but they can be absorbed better in the human body due to the lower viscous drag.

12. The acid value of our samples i.e. the amount of free fatty acids lies within the range of 0.5 and 3 mg KOH/g which is well within the limit of 0.6 and 10 mg KOH/g considered ideal for human consumption.
13. The Iodine value of the hemp seed oils lie within the range of 150 and 170 which is in line with previous published reports and shows the high amount of unsaturation in the oils making it suitable for human consumption.
14. The maximum induction time of 2.56 hours was reported for cold pressed hemp heart oils whereas the minimum of 0.43 hour was reported for US oils. The low induction times for the oils showed that the degree of saturation is less making it an excellent source of nutrition.
15. The acid value, iodine value and oxidative stability results showed that the oil obtained by each of the extraction techniques was not degraded and was of improved quality.

### **Objective 3**

The conditions of extraction yielding maximum oil from each of the extraction techniques used were compared to find the most efficient method in terms of reaction time as well as % yield. After assessment of the physicochemical characters of the oils, the areas of application of our oils were recommended.

1. Among the different extraction techniques used, MPSE oils and MW oils produced the maximum extraction yield of 54 % (w/w) whereas cold pressed seeds produced the minimum extraction yield of 41 % (w/w).
2. The maximum extraction rate of 0.448 g oil/min extraction was observed for MW extraction.
3. Different FA and antioxidants have been analyzed in the extracted hemp oils obtained by different techniques as discussed above which have antioxidant properties and are also beneficial for the human health.

4. Furthermore, the Acid Value and Oxidative stability of the hemp heart oils are low, and the Iodine value is high proving that the amount of free fatty acids as well as the degree of saturation in the oil is low making it a good consumable product.
5. Based on the above results, it can be concluded that microwave extraction is the best extraction technique in terms of extraction yield and efficiency but not in terms of quality (from preliminary quality studies).
6. On the other hand, although the extraction efficiency is not that high, the quality of oil produced by cold pressing is better compared to the other extraction techniques as is evident from the IV, AV and induction times.
7. Overall, it can be concluded that different extraction techniques will influence the chemical composition of the hemp seed oils from a nutritional aspect as well as for co-extraction of hempseed phytochemicals of interest to the higher value nutraceutical and pharmaceutical industries.

## **5.2. Recommendations for future work:**

1. As the hemp seed meal/cake is highly rich in proteins and dietary fibers, further extraction and analysis of the proteins can be performed in order to increase the nutritional value of the seeds. The use of the hemp seed residual meal as animal fodder or as an aquaculture ingredient can also be explored.
2. All the experiments conducted in this research were at a laboratory scale. Hence, further studies on pilot scale processing is recommended for the techniques to be used on an industrial level.
3. The positive and negative aspects of both M.W extraction and C.P. have been concluded upon results based on laboratory experiments. In order to apply any of these to the industry, thorough

economic evaluations need to be performed on the energy consumption during the experiment and also optimize the energy consumed to increase its cost effectiveness.

4. Different FA, antioxidants and minor compounds have been identified in our oil samples whose health benefits have been recommended in this study. This particular topic needs further exploration and research and clinical trials need to be performed before we can actually understand the nutritional and/or health benefits of the oil.
5. In our research, the importance of the oil in the higher value nutraceutical and pharmaceutical industries have been highlighted. There is further scope to use this oil in the cosmetic and body care industries.
6. In our studies, dehulled hemp seeds (hemp hearts) have been utilized. Extraction experiments with whole seeds can be a future scope of research.
7. Further investigations should be made on quality studies of MW extracted oils as it cannot be categorically concluded to be inferior in quality and even if there is any deterioration in the quality, then a thorough comparison should be established with cold pressed oils.

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